THE JOURNAL OF EXPERIMENTAL MEDICINE

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INTRODUCTION.

THE need of an American journal devoted especially to the publication of original contributions to the medical sciences has been keenly felt for some time. Our investigators in these departments have hitherto been obliged either to publish their scientific papers, often in condensed form and imperfectly illustrated, in periodicals devoted mainly to the practical branches of medicine, or to send them, as has become increasingly the custom, to various scientific journals of Europe. The result has been that we ourselves, and in still greater measure foreigners, do not know where to look for many of the widely scattered contributions of American workers to physiology, pathology, bacteriology, and other medical sciences.

In consequence of the absence of such a journal as is here contemplated there has been a lack of the close contact and free interchange of ideas, both between our own workers and between American and foreign investigators, which are fruitful in stimulating scientific research and in securing both here and abroad due consideration of work done in this country.

An American journal devoted exclusively to the publication of original work in the medical sciences should aid materially that rapid advancement of scientific medicine which has been evidenced during recent years in this country by the elevation of the standards of medical education, by the establishment of well-equipped laboratories for the various medical sciences, by the increase in the num-

ber of well-trained workers to make use of these greater opportunities, by the growing number and importance of the contributions of American medical investigators, and by the wider appreciation and more generous support of scientific medical work.

It is believed to be for the interest both of the scientific worker and of the medical public that the first journal of this character established in this country should, like similar pioneer journals and some existing journals in other countries, embrace within its scope various allied medical sciences.

The title, *The Journal of Experimental Medicine*, has been selected in preference to a more specific but cumbersome designation in order to indicate broadly that the *Journal* is not intended for purely clinical contributions. In medical, no less than in other, sciences the great advances of modern times have been owing to the method of experiment combined with that of exact observation. The term "Experimental Medicine" in the title is not intended to imply that the publication of investigations based upon observation is not within the province of the *Journal*. Anatomical contributions both to physiology and to pathology are included within its scope.

It is intended that contributions in physiology and physiological chemistry shall be a prominent feature of the *Journal*. Physiology is the foundation of scientific medicine, and the combined representation in the *Journal* of physiology and pathology emphasizes their intimate union and mutual relations.

Pharmacology regarded as an experimental science, closely allied by its methods of work and by many of its problems to physiology, finds appropriate place in a journal of experimental medicine.

Studies in general pathology and pathological anatomy will constitute a large and important part of the material of the *Journal*.

While the broad and diversified field of modern hygiene can not be embraced in its entirety within the domain of the *Journal*, there are many directions of experimental research in this department which may appropriately be included.

The epochal discoveries of the causal relations of micro-organisms to infectious diseases have opened a fruitful and important field of research. No medical science is to-day cultivated more zealously and with more significant results than that of bacteriology, which will occupy a position in the *Journal* commensurate with its great importance.

While purely clinical observations do not come within the domain of *The Journal of Experimental Medicine*, its pages will be open to the publication of investigations such as are now made in some hospitals and clinical laboratories. Methods of the laboratory, microscopical, chemical, and bacteriological, have found fruitful application in the study of disease in the living patient, as is shown by the results of such hospital studies as those on the blood, on metabolism, on gastric contents, on the presence and significance of micro-organisms in infectious processes. It is in such directions as those here indicated that medicine appears as one of the departments represented in the *Journal*.

Although the subjects embraced within the scope of this *Journal*, as thus outlined, are highly specialized, still they have many interests in common as to their methods and problems and purposes, and it is believed that their alliance in the present *Journal* will prove of mutual advantage, will promote scientific medicine, and is best adapted to existing conditions in this country.

With the editor will co-operate a board of associate editors who are leading representatives of their special departments in the United States and Canada. The assistance of a large number of distinguished American collaborators who are active workers in the departments represented in the *Journal* has been secured. The gratifying success in obtaining this general co-operation, and the many spontaneous expressions of good will and approval which have already been received, inspire confidence that *The Journal of Experimental Medicine* will be truly representative of scientific medicine in the United States and Canada.

THE EDITOR.

OBSERVATIONS CONCERNING THE BACILLUS AEROGENES CAPSULATUS.

By WILLIAM H. WELCH, M. D.,
AND
SIMON FLEXNER, M. D.
(*From the Pathological Laboratory of the Johns Hopkins University and Hospital.*)

In November, 1891, one of us (Welch *), at a meeting of the Johns Hopkins Hospital Medical Society, reported with demonstrations the discovery of a gas-forming bacillus found in the emphysematous tissues and blood of a man dead of aneurism of the aorta. In July-August, 1892, Welch and Nuttall published a full report of this case and described the characters of the bacillus, for which they proposed the name *Bacillus aerogenes capsulatus*. Since this report there have been published, both in this country and in Europe, additional observations of the occurrence of this bacillus, and we have had the opportunity of studying a considerable number of cases in which this organism was found. We purpose in the present article, upon the basis of these published reports and of our further observations, to consider the distribution and characters of this micro-organism, and more particularly its relations to various affections and conditions of human beings.

We must refer to the original paper of Welch and Nuttall for the full report of the case in which the bacillus aerogenes capsulatus was first found, for the detailed description of the morphological and cultural characters and the effects of intravenous inoculation of this bacillus, and for the deductions which were then drawn as to the probable relation of the bacillus to conditions characterized by the presence of gas in the blood and tissues. Here we shall give a summary of some of the salient points contained in this paper.

* The references to literature are arranged alphabetically at the end of this article.

The patient was a mulatto man, aged thirty-eight, with tuberculosis and with aneurism of the ascending aorta which had perforated by a small opening through the anterior thoracic wall, and which had given rise to repeated external hæmorrhages, the last occurring two days before death, which was sudden. No subcutaneous emphysema was noted during life. The autopsy was made in cool weather, eight hours after death, the body being still warm. There was no odour, greenish colour, or other evidence of post-mortem decomposition. There existed emphysema of the subcutaneous tissues of the greater part of the body. The blood vessels and heart contained abundant gas bubbles. The blood was lake-coloured, and the lining membrane of the heart and blood vessels showed diffuse imbibition of blood-colouring matter. There was gas in the blood-stained serum in the pericardial sac and in the peritonæum. Gas bubbles were present abundantly in the internal tissues, notably in the myocardium, the liver, spleen, and kidneys, these organs presenting the appearances described by German writers as those of *Schaumorgane.* Gas bubbles were numerous in the discoloured, whitish, ante-mortem coagula which nearly filled the aneurismal sac. The gas burned with a pale bluish, almost colourless flame, a slight detonation being heard at the moment of ignition.

Upon microscopical examination of the organs there were observed around masses of bacilli frequently, but not always, disappearance of the nuclei and degenerative changes in the cardiac muscle cells and the epithelial cells of the liver and kidney, especially in the neighbourhood of gas blebs, in the walls of which the bacilli were often densely accumulated.

By examination of cover-slip preparations a single species of bacillus was found in very large numbers in the blood and in all other situations in which gas was present. Aerobic cultures from the blood and organs remained sterile. Anaerobic cultures yielded numerous colonies of the same bacillus found in cover-glass preparations from the fresh blood and organs, no other species developing.

The morphological and cultural characters of the bacillus were

described in full detail. Only the leading characters will here be recapitulated.

The bacillus is non-motile, straight or sometimes slightly curved, variable in size, but averaging about the thickness of the anthrax bacillus and 3 to 6 μ in length, with adjacent ends slightly rounded or sometimes square-cut; occurs singly, in pairs, in clumps, and sometimes in short chains, less frequently in threads and long chains; and stains readily with the ordinary aniline dyes, including Gram's and Weigert's fibrin stain, either uniformly or with small unstained spots, less frequently with isolated deeply staining granules. Capsules, although not constant, were frequently demonstrated, especially by Welch's method for staining capsules, in specimens from the animal body and sometimes from agar cultures. No spores were found either in the animal body or in cultures.

The bacillus grows upon all ordinary culture media—rapidly at body temperature, slowly at temperatures as low as 18° to 20° C. It is anaerobic, no growth occurring on the surface of solid media or in ordinary fluid cultures in test tubes exposed to the air. There is growth in the depth of solid media exposed to the air. Under anaerobic conditions growth occurs both upon the surface and in the depth of solid media.

Gas is produced in all cultures containing fermentable material. In our plain nutrient agar or gelatin there is usually some formation of gas,* and in media to which one per cent of glucose has been added there is at body temperature a rapid and very abundant development of gas.

The colonies in agar are grayish-white to a more opaque white or brownish-white colour by transmitted light, sometimes with a central darker dot. At the end of twenty-four hours they do not

* As has been pointed out by Theobald Smith, the amount of sugar in ordinary meat bouillon varies with the character of the meat and the length of time it has been kept. In some meat and meat kept a considerable time there may be no sugar. The gas produced by fermentation of carbohydrate, therefore, varies in amount in different lots of media made with plain bouillon, and may be absent altogether. These facts will explain many of the discrepancies of statement of different observers as to the production of acid and of gas by various bacterial species in nutrient media to which sugar has not been added.

exceed 0·5 to 1 millimetre, but they may subsequently attain a diameter of 2 to 3 millimetres, or even larger. Those in the depth are spheres or ovals, generally more or less flattened, usually, but not always, with irregular contours, the irregularity being due to knoblike or feathery projections. No putrescent or distinctive odour could be detected in the cultures, except sometimes a foul odour when masses of agar culture were crushed in a little bouillon.

In gelatin cultures there was observed a slow and limited liquefaction of the medium, manifested by a settling of the growth and by slight displacement of the gas bubbles in changing the position of the tube.

The bacillus produces at first diffuse cloudiness of sugar bouillon; later the fluid becomes clearer, with an abundant whitish, more or less viscid sediment. In twenty-four to forty-eight hours milk is coagulated, the clot being generally firm, retracted, and furrowed with the marks of gas bubbles. In anaerobic potato cultures there is either no visible growth or a thin, moist, grayish-white growth on the surface and abundant formation of gas in the fluid accumulated about the bottom and sides of the potato. In ascitic fluid there is abundant growth with formation of gas bubbles.

The vitality of the cultures is of very variable duration, according to the character of the medium and the mode of cultivation. Cultures in sugar bouillon in Liborius tubes filled with hydrogen were found to be dead in three days or less. On the other hand, sugar-bouillon cultures in Buchner's jar may survive four months and longer. Cultures in sugar media are shorter lived than those in plain media. Cultures in tubes hermetically sealed after two or three days' growth survived several months. The thermal death point, determined by Sternberg's method, was found to be 58° C. for ten minutes.

Experiments upon animals were made chiefly to elucidate the relation of the bacillus to the conditions found at the autopsy upon the patient. With one important exception, to be mentioned subsequently, it was determined that the bacillus is not pathogenic for rabbits when introduced directly into the circulation.

If the animal be killed immediately or soon after the intravenous injection of 0·5 to 1 cubic centimetre of bouillon culture, after eighteen to twenty-four hours at the temperature of 18° to 20° C., and after four to six hours at temperatures from 30° to 35° C., the bacilli are found abundantly, together with great formation of gas, in the blood vessels and organs. The viscera present the typical characters of *Schaumorgane*. All of the conditions relating to the bacilli and their production of gas which existed at the post-mortem examination of the patient were reproduced experimentally in animals.

When the bacilli are introduced at one place in the vascular system soon after death, they develop in the course of the vessels into which they have been introduced, and the time required for them to appear with gas formation throughout the vessels is much longer than when they have been distributed throughout the circulation just before death.

These experiments upon animals were considered to favour the view that in the patient the bacilli had entered the circulation before death, and probably primarily from without through the opening in the thoracic wall into the aneurismal sac. The multiplication of the bacilli in the vessels and the formation of gas were regarded as occurring mainly post mortem.

The bearing of these observations upon various conditions of the body characterized by the presence of gas was considered, particularly upon the reported cases of death from the entrance of air into the circulation and upon cases in which gas, unassociated with ordinary cadaveric decomposition, was found in the blood vessels after death without the existence of any opening through which air could enter the circulation.

In view of the positive result of an intravenous inoculation of a pregnant rabbit with a culture of the bacillus aerogenes capsulatus, especial emphasis was laid upon the possible relationship of this bacillus to the reported deaths attributed to the entrance of air into the uterine veins in the puerperal state, after abortions, and after injections into the uterine cavity. As the explanation of these cases suggested by Welch and Nuttall has since been confirmed, we will

introduce here the protocol of the original experiment upon the rabbit.

Experiment 9.—Pregnant rabbit, 1 cubic centimetre of sugar-bouillon culture (ninth generation), forty-eight hours old, into ear vein. Rabbit was found dead twenty-one hours after the inoculation, the body being still warm, although stiff. The death was sudden. The rabbit was alive half an hour previously, and apparently in good condition. The autopsy was made six hours after death. By this time the body, which has been in a room at the temperature of about 18° C., is much swollen, the abdomen distended, and emphysematous crackling can be felt over the abdomen and the lower part of the thorax. Bloody fluid containing gas bubbles oozes from the vagina. On opening the peritoneal cavity gas escapes freely, which burns with a colourless flame, a slight explosive sound being heard upon first applying the match. This cavity contains blood-stained fluid. Blood vessels, both veins and arteries, contain gas. The heart cavities on both sides contain soft dark clots with gas bubbles. Spleen is somewhat enlarged, soft, and dark red. Liver does not contain gas blebs, but gas is present in the hepatic vessels. The abdominal vena cava is blown up with gas. The uterus contains six embryos about three centimetres long. The sacs of the uterus corresponding to these embryos are greatly distended with gas. Two of the sacs are more distended than the rest, their walls are very thin, contain gas bubbles, and look as if they were just ready to burst. The embryos in these two sacs are macerated, dark livid red, partly destroyed and smaller than the others, which are intact. The placentæ contain gas bubbles in large amount.

Cover-slip preparations show an enormous number of characteristic capsulated bacilli in the distended uterine sacs, and a large but not so great number in the liver, spleen, heart, and blood vessels. Cultures from the uterus, spleen, and left ventricle are pure of the typical gas-forming bacillus which was used for the injection.

It seems probable from the autopsy that two of the embryos in the uterus were already dead when the injection was made, and that in these embryos and the part of the uterus containing them the bacilli were able to gain a foothold and develop. The extent of development of the bacillus and of gas was much greater than is observed at so short a period after death (six hours) when the animal is killed immediately or shortly after intravenous injection of the cultures and is kept at the temperature of the room.

Inasmuch as we now know, from observations made since Welch and Nuttall's publication, that the bacillus aerogenes capsulatus is a widely distributed organism, of not infrequent occurrence in the human

body, and that it may be associated with various pathological conditions, we have searched through the literature in order to find whether it may not have been recognised by earlier observers. It seems highly probable that it must have been seen before its isolation and description by Welch and Nuttall.

It is not a little remarkable that of the considerable number of reported cases of suspected entrance of air into the blood vessels, none seem to have been previously the subject of bacteriological study. For some of the cases in which gas was found at the fresh autopsy widely distributed in the blood vessels and tissues several writers have recognised the difficulties involved in the usual explanation that the gas was atmospheric air or the result of ordinary cadaveric putrefaction. When this condition was found associated at autopsy with an emphysematous phlegmon or gangrene, some observers have recognised the specific bacterium causing the latter affection during life as also the cause of the development of gas in the blood and internal organs either during life or after death.

In the various reports of cases of gas in cysts, joints, serous cavities, urinary passages, and uterus we have been unable to find at a date prior to that of Welch and Nuttall's publication any description of a bacterium resembling the bacillus aerogenes capsulatus.

On the other hand, we have little or no doubt that in the cases reported before 1892 under the names malignant œdema, emphysematous or gaseous gangrene, gaseous phlegmon, emphysematous cellulitis, gangrenous septicæmia, gangrène gazeuse foudroyante, etc., the bacilli usually described as those of malignant œdema were in some instances identical with the bacillus aerogenes capsulatus. We shall consider subsequently in this article the possible relationship of these bacterial species, but in this connection it may be said that when in the class of cases under consideration the identification of an anaerobic bacillus, as that of malignant œdema, is based solely upon general morphological resemblances and similar pathogenic effects, without determination of motility and development of spores, the bacillus in question may, in the light of our present knowledge, equally well be regarded as the bacillus aerogenes capsulatus.

There are not a few cases which fall under this latter category, but in these the characters of the bacilli have not been described with such detail as to permit their positive identification. On this account it does not seem to us worth while in this article to attempt to disentangle as to this matter the generally unsatisfactory and incomplete descriptions of supposed malignant œdema bacilli in this group of diseases in human beings.

The case reported in 1888 by Bremer as one of malignant œdema may serve as an illustration of the cases which we should regard as probably referable to the bacillus aerogenes capsulatus. In this case, at an autopsy made seventeen hours after death of a woman who developed as a sequel to an abortion emphysematous swelling of the right pectoral region and adjacent arm, Bremer found most extensive development of gas in the blood vessels, the tissues, and the thoracic and abdominal viscera, with an enormous number of bacilli, devoid of spores, in the uterus, blood, organs, and serous cavities. The bacilli were said to resemble the bacillus œdematis maligni. Nothing is said as to motility or threadlike forms of the bacillus, nor was it obtained in culture. The inoculation of guinea-pigs with serosanguinolent fluid containing the bacilli was fatal in twenty-four hours, with lesions said to be like those of malignant œdema.

Of cases of gaseous phlegmon reported before 1892 in which anaerobic bacilli somewhat resembling the bacillus of malignant œdema, but not identified with it, were found, those of F. J. Rosenbach, Wicklein, and Levy have received special consideration by E. Fraenkel in his monograph *Ueber Gasphlegmonen*. He comes to the conclusion that only the bacillus described by Levy in 1891 is probably identical with the bacillus aerogenes capsulatus.

Rosenbach's bacillus formed spores and he was unable to cultivate it. The bacillus emphysematis maligni of Wicklein was motile, grew in long, jointed threads, formed spores both in cultures and in the animal body, and was pathogenic only for guinea-pigs. In spite of the contrary conclusion reached by Wicklein, Fraenkel is inclined to identify this bacillus with that of malignant œdema.

The bacillus isolated by Levy in 1891 from a gaseous phlegmon

of the pelvis and thigh of a puerperal woman was anaerobic, gas-producing, non-motile, short, small, delicate, stained by Gram, grew in long threads and chains of thirty and more members, and formed anthraxlike colonies. It grew only at body temperature, and was of so short viability that it was cultivated only in the first generation. No experiments upon animals were made. The rapid healing of the abscess was attributed by Levy to the short viability of the bacillus. In a subsequent article published in 1895 Levy declares this bacillus to be identical with Fraenkel's bacillus phlegmones emphysematosæ, but we do not consider that Levy's original description of this bacillus suffices for such identification, especially in the absence of experiments on animals; nor can his description of the bacillus as short, small, and delicate, ("*kurze, kleine, feine Bacillen*"), and as incapable of growth below the body temperature, be reconciled with the characters of the bacillus aerogenes capsulatus.

As stated by Welch and Nuttall, the bacillus described by E. Fraenkel, in 1889, in a case of gastritis emphysematosa may be identical with our bacillus, but it was studied only on hardened specimens of the stomach.

It would not be surprising if the bacillus aerogenes capsulatus should be found among the considerable number of anaerobic gas-producing bacilli which have been isolated from the soil, the fæces, decomposing infusions of animal or vegetable matter, and other sources. Most of these bacilli are motile and sporogenic. Many differ from each other only in such minor characters that their separation into distinct species is difficult or impossible. As there is reason to believe that the bacillus aerogenes capsulatus may exist in the soil, we have examined especially the descriptions of the various bacilli isolated from this source by Liborius, Lüderitz, Sanfelice, and others. But we have not found a bacillus described which we are able to identify in all respects with our bacillus, although we can by no means exclude in every case the possibility of identity. The same statement applies to the several varieties of malignant œdema, pseudo-œdema, and Rauschbrand bacilli which have been described. There is usually some point regarding motility or spore formation

or conditions of growth or odour or other cultural character or the result of or absence of animal experiment which prevented such identification. We shall have something to say subsequently as to the value of such points as these in the differentiation of species among this group of anaerobic bacilli.

The first observations of the bacillus aerogenes capsulatus following the report of Welch and Nuttall were made by E. Fraenkel, who published, in January, 1893, a preliminary report, and later in the same year a monograph concerning gaseous phlegmons. Fraenkel makes no mention of the previous discovery of the same organism by Welch and Nuttall, and doubtless was unacquainted with their work. To Fraenkel belongs the credit of demonstrating the causal relation of the bacillus aerogenes capsulatus to the affection called variously gaseous phlegmon, emphysematous cellulitis or gangrene, gangrène gazeuse, etc.

Fraenkel in these communications reported the results of his examination of four cases of gaseous phlegmon, in one of which the gas bacillus was found unmixed with other bacteria, whereas in the remaining three it was mixed with other bacteria, chiefly pyogenic cocci. In the case of pure infection with the gas bacillus there was no suppuration in any part of the enormous emphysematous swelling of the right lower extremity. On incision a turbid brownish fluid, without offensive odour and containing gas bubbles, escaped. The muscles were completely disintegrated into an opaque reddish-gray pulp filled with bubbles of gas. It is noteworthy that in two of the cases the gaseous phlegmon followed hypodermic injections, in one case of camphor oil and ether and in the other of a dilute solution in water of sulphuric acid and chloride of morphine. The latter was the case of unmixed infection and pursued a rapidly fatal course, death occurring two days after the injection. Three of the cases terminated fatally; in one the issue is not stated. In none of the cases was gas found at autopsy in the internal organs or blood vessels.

The bacillus isolated by Fraenkel from these cases corresponded in all essential morphological and cultural characters with the bacil-

lus aerogenes capsulatus. To it Fraenkel gave the name of bacillus phlegmones emphysematosæ. As regards the formation of spores, he states that this is inconstant and exceptional, and was observed by him only in a few of the bacilli in cultures in one lot of agar containing formate of sodium. Spores were not noticed in other agar cultures, even when the medium was made according to the same formula with formate of sodium. A clear zone was noticed around bacilli from the animal body and from surface agar cultures, but no capsule was stained. The probable identity of Fraenkel's bacillus of gaseous phlegmon with the bacillus aerogenes capsulatus was recognised by Mann, in an article published in February, 1894, who worked in our laboratory, and by one of us (Welch) in a published article on The General Bacteriology of Surgical Infections. Through the kindness of Dr. Fraenkel we have been enabled to examine cultures of his bacillus which he has sent us, and we have found this organism in every respect identical with that described by Welch and Nuttall. This identity is admitted in a recent communication by Goebel, whose observations come from the same hospital in Hamburg with those of Fraenkel, and in a personal communication from Dr. Fraenkel to one of us (Welch) the same opinion is expressed.

As the experiments of Welch and Nuttall were conducted to throw light upon the relationship of the gas bacillus to conditions found in their patient, so those of Fraenkel were for the purpose of determining its relation to gaseous phlegmons. Conducted thus from different points of view, these two sets of experiments are mutually complemental, and taken together they have established the main facts pertaining to the effects of inoculation of animals with this micro-organism. Fraenkel's experiments were made by inoculation of mice, rabbits, and guinea-pigs. No result followed subcutaneous injection of cultures into mice. Rabbits survived intravenous inoculation without any apparent disturbance. Sometimes, but not constantly, the subcutaneous inoculation of rabbits was followed by local œdema with necrosis of the overlying skin, from which the animal recovered. The presence of gas in the swelling is not mentioned.

In guinea-pigs, however, subcutaneous inoculation of cultures of the bacillus caused a process essentially identical with the condition observed in the human patients. The first effect was a painful infiltration of the tissues with a blood-stained fluid extending from the site of inoculation. In more than half of the cases gas appeared in the swelling within the first twenty-four hours, in the remainder at a later period. Death may occur in twenty-nine to thirty-six hours after inoculation or at a later period. In a considerable number of cases the skin bursts over the infiltrated area, and then after prolonged necrosis and ulceration recovery by cicatrization may occur. There was no actual suppuration unless pyogenic bacteria were mixed with the gas bacillus. At autopsy the muscles of the affected area were converted into a soft, pulpy mass and blood-stained fluid infiltrated the part, which contained gas bubbles in large number. There may be direct extension of the inflammation to the peritoneal and pleural membranes. The bacilli were found only in the actually inflamed and necrotic parts, but here in enormous number, occurring in the form both of rods and of threads.

The next publications concerning the bacillus aerogenes capsulatus were those of Graham, Steward and Baldwin, and of P. Ernst, both of these articles appearing in August, 1893. The case reported by Graham, Steward and Baldwin is of extraordinary interest. The patient was a labouring woman, married, aged thirty-five. About fourteen hours before death the woman, who had previously been well, was seized with what she described as a severe chill, which lasted more than four hours. This was followed by pain in the region of the uterus and ovaries and by vomiting and purging.

She was first seen by Dr. Steward at 1 P. M., about five hours after the onset of the first symptoms. She was then extremely restless and suffering from "distress and pain" in the lower abdominal region. There was a slight flow of bright-red blood from the uterus. Abortion was suspected. The pulse was about 120. Morphine was administered hypodermically. The patient when seen at 3.30 P. M. appeared much relieved and dozed off to sleep. She was not visited again by Dr. Steward until half past nine in the evening, when he

found her "emphysematous from the top of the head to the soles of the feet." Emphysematous crackling could be felt over the entire surface of the body. There was a diffuse blood-red colour of the whole surface of the body. The skin was smooth. The husband said that the swelling, redness, and crackling began about 5.30 P. M. The pulse was weak; the breathing somewhat rapid. We quote from Dr. Steward's report: "The patient said that she was not now suffering so much pain, but that she felt bloated, with a sense of numbness over the entire surface of the body. She had not passed urine since morning. I introduced a catheter and drew only a few drops of bloody urine. Noticing that her breathing was becoming very short and quick, I felt for the pulse. It was gone. The heart had stopped beating. The woman was dead. There was no death struggle. The patient was entirely conscious and talking to within three minutes of death. There was no change in the ruby appearance of the skin at the approach or instant of death."

The emphysematous condition increased so rapidly after death as to alarm the undertaker. Eighteen hours after death the body was bloated to more than double its natural size. The autopsy was made about twenty hours after death. The uterus gave evidences of a recent abortion at about the third month. It contained bloody fluid and placental *débris*, and its interior presented suspicious marks, but no positive evidence of criminality was obtained. The vessels, tissues, and organs were enormously distended with gas, which ignited with an explosive sound, and was determined by chemical examination to be largely hydrogen. The bacteriological examination made by Graham showed in the blood in very large number and pure culture bacilli, which were identified by microscopical examination, cultures, and the inoculation of animals as the bacillus aerogenes capsulatus. Dr. Graham kindly sent one of us (Welch) microscopical specimens and photographs of the bacillus, which in the characters thus revealed we found to be indistinguishable from our bacillus.

This case was recognised by Baldwin and Graham before the autopsy as probably referable to the bacillus previously described

by Welch and Nuttall, and reference is made by Baldwin to the brief report of a case presenting similar post-mortem appearances in the same journal of October, 1892.

The significance of the remarkable case reported by Graham, Steward and Baldwin is that it affords positive evidence that the gas bacillus may invade the blood and produce gas widely distributed over the body during life. The development of extensive emphysema of the entire subcutaneous tissue of the body in this case during life and within the space of four hours can not well be explained otherwise than upon this supposition. The portal of entry of the bacillus was manifestly the uterus, which presented unmistakable evidences of a recent abortion, possibly artificially produced by the insertion of some instrument. This case therefore confirms the explanation suggested by Welch and Nuttall of deaths previously reported as due to the entrance of air into the uterine veins after abortions.

The attention of P. Ernst was arrested especially by the formation of foam upon the cut surface of the liver at autopsy, this foam being due to the development of gas by the bacillus aerogenes capsulatus. He reported in August, 1893, two observations of this foaming liver (*Schaumleber*). These cases were observed prior to the publication of Welch and Nuttall, which led Ernst not to delay longer the report of his own studies upon the same subject.

The first case was that of a woman who developed putrid endometritis and septic peritonitis after removal of a macerated four months' fœtus, the uterus, through doubt as to the diagnosis, having been previously curetted, sounded, and tamponed. No development of gas in the tissues was noted during life. At the autopsy, made only three hours after death, the entire uterine wall crepitated from the contained gas. Coagula in the right iliac vein and conus pulmonalis contained gas. Gas was found also in the myocardium, and especially in the liver, from the cut surface of which gas oozed up and in a few hours formed a layer of foam several centimetres thick.

A similar condition of the liver was observed at an autopsy made

eighteen hours after death upon a case of peritonitis resulting from traumatic perforation of intestine in a hernial sac. The patient died four hours after a hernio-laparotomy. Green discoloration of the liver and other parts indicated advanced cadaveric decomposition. Gas was found in the liver and gas blebs in the intestinal mucosa.

The organism found in these two cases was a non-motile, anaerobic bacillus corresponding in its essential features with the bacillus aerogenes capsulatus, with which it was identified by Ernst. Rods exceeding 5 μ in length were met only very exceptionally. Capsules were not stained and growth in gelatin at room temperature was not obtained, but it is not clear how thoroughly examinations were made as to these points. Only a few experiments upon animals were made. It is noteworthy, in view of the negative results of Fraenkel, that Ernst succeeded in killing two mice by subcutaneous inoculation with the bloody fluid from the livers, the characteristic bacilli being found, at least in one of the mice, in the heart's blood, liver, and spleen. Subcutaneous inoculation of a guinea-pig with hepatic fluid from the first case caused death in less than twenty-four hours, with foul-smelling gangrene of the skin and sanguinolent œdema, which contained a variety of cocci and bacilli besides the typical gas bacillus. The guinea-pig inoculated with material from the second case survived. A rabbit inoculated into the ear vein with blood rich in bacilli from the first case became manifestly ill and voided turbid bloody urine containing the characteristic bacillus and long threads, which were regarded as possibly belonging to the same species. Contrary to expectation, the rabbit recovered.

Ernst describes with much detail the histological changes in the liver, these being characterized, as pointed out by Welch and Nuttall, by disappearance of nuclei and degenerative changes around masses of bacilli. The characters of these alterations are believed by Ernst to indicate the lodgment of the bacilli in the liver during life. The development of gas holes in the liver, however, he regards as post mortem.

In February, 1894, Mann reported a case of emphysematous gangrene of the hand due to the bacillus aerogenes capsulatus associated with the streptococcus pyogenes. The patient was in Dr. Tiffany's clinic at the University of Maryland. The affection began as a small black blister on the palmar surface and near the end of the left ring finger. The entire hand became swollen and œdematous, and upon palpation emphysematous crackling could be felt up to the wrist. The temperature rose to 105·5° F. and the pulse to 130. Free incisions were made and the hand placed in a hot sublimate bath. Recovery, with loss of the gangrenous extremity of the middle finger, took place.

Aerobic cultures were pure of the streptococcus pyogenes. By means of anaerobic plate cultures the bacillus aerogenes capsulatus was isolated in purity and was fully identified by Mann, who kindly submitted to me his cultures. Mann calls attention to the similarity of the lesions produced by the bacillus œdematis maligni and the bacillus aerogenes capsulatus, which he regards as distinct species.

In May, 1895, Levy, under the title of pneumothorax without perforation, published an observation of the bacillus aerogenes capsulatus. In a patient forty-eight years old, after repeated aspirations of clear fluid from the left pleural cavity, there developed pneumothorax. The slightly turbid serous fluid withdrawn by aseptic aspiration eight days after the first appearance of the signs of pneumothorax, as well as the opaque fluid removed a week later by resection of a rib, furnished in anaerobic cultures a short, thick, plump, non-motile, gas-producing bacillus, devoid of spores, staining by Gram, occurring usually as a diplobacillus, but not infrequently also as jointed threads, and growing both at body and room temperatures. Bouillon cultures possessed a peculiar viscid consistence, such as is customary with capsulated bacteria, but no distinct capsule could be demonstrated. Subcutaneous or intraperitoneal inoculation of guinea-pigs caused reddish serous exudate filled with gas bubbles at the site of inoculation. In one guinea-pig a few bacilli were found in the blood as well as in the exudate.

Levy interprets the case as primarily one of tuberculous pleurisy

to which in some unknown way the gas bacillus gained access and caused during life gas formation without perforation. At the autopsy by von Recklinghausen a fibrous mass the size of an egg containing caseous foci was found in the upper part of the right lung, and a small caseous focus was found in the lower lobe of the left lung without rupture of the pleura. The possibility of a healed perforation of the pleura could not be positively excluded, but this was considered by von Recklinghausen to be improbable. Unfortunately, nothing is said as to the results of aerobic cultures, nor whether other bacilli than the one described were present, nor as to the direct microscopical examination for bacteria of the pleuritic exudate.

Levy identifies the bacillus isolated from this case with that obtained from the case of rapidly healing gas abscess already cited, although the bacillus from the latter case was described as small and delicate and incapable of growth at ordinary temperatures and was only imperfectly studied. He doubtless correctly identifies the bacillus from the case of pneumopleuritis with Fraenkel's bacillus of gaseous phlegmon. With the exception of his own first doubtful observation of this bacillus and of Fraenkel's studies, he makes no mention of any of the other observations of the same bacillus.

In July, 1895, Goebel* published an article concerning the bacillus of *Schaumorgane*, confirming the results of Welch and Nuttall, and demonstrating that this bacillus is identical with the bacillus phlegmones emphysematosæ of Fraenkel. He cultivated the bacillus from three cases at autopsy. The first was a case of pyelonephritis consecutive to papilloma of the bladder and abscesses of the prostate and seminal vesicles. Gas blebs were found in the heart muscle, liver, spleen, adrenals, and stomach. Around many, but not all, of the blebs in the heart and liver the cells were necrotic. Necroses were not present in the spleen around the gas holes.

* This article of Goebel is described as an extract from an elaborate paper which is to appear in the *Jahrbücher der Hamburgischen Staatskrankenanstalten*. In the latter Goebel promises a detailed consideration of the differential diagnosis of the bacillus aerogenes capsulatus from other similar anaerobes (malignant œdema, *Rauschbrand*, etc.), and further conclusions to be drawn from his observations. This volume of the *Jahrbücher* has not yet appeared (December, 1895), so far as we have been able to learn.

The second case was one of enlarged prostate which had been catheterized. There existed general arteriosclerosis, pulmonary emphysema, lobular pneumonia, and embolism of branches of the pulmonary artery. Numerous gas blebs were found at autopsy beneath the mucous membrane of the urinary bladder. Necroses were absent from the walls of these blebs.

The third case was one of pyæmia following endophlebitis of the cutaneous veins of the left foot. Beneath the gastric and intestinal mucosa were gas blebs resembling those in the preceding case, except that necroses were present in the neighbourhood of the blebs.

The gas vesicles in all of these cases contained numerous bacilli in their walls and interior. The characters of the bacillus isolated from these cases correspond in all particulars with the bacillus aerogenes capsulatus of Welch and Nuttall and the bacillus phlegmones emphysematosæ of Fraenkel. Capsules were stained by Goebel around bacilli from the animal body, but apparently only once around bacilli from cultures. The experiments of Welch and Nuttall were repeated with identical results both with the bacillus isolated from foaming organs and with that from gaseous phlegmons. Necroses were absent from the walls of gaseous blebs which formed in the organs of rabbits which had been killed a few minutes after intravenous injection of cultures. Spore formation was observed only in one culture, this being a forty-eight-hour stab culture in sodium-formate agar. Liquefaction of gelatin with cultures from the first case was slow and limited; with those from the second it was rapid and marked. Subcutaneous inoculation of guinea-pigs produced the same effects as those described by Fraenkel in his monograph on gas phlegmons.

In the first and third cases it was considered, in view of the presence of necroses, that the gas bacilli invaded during life the organs containing the blebs at autopsy, whereas, in the second case, the invasion of the bacillus is regarded as post mortem.

Passow has recently reported a case of gaseous phlegmon of the right shoulder joint which was observed in November, 1893. Three

weeks after a superficial wound of the hand which healed slowly by suppuration the patient developed septicæmia with purulent panophthalmitis, and a few days later an emphysematous swelling around the right shoulder joint and extending down the arm. Upon incision, stinking, brownish, non-purulent fluid containing gas with the odour of sulphuretted hydrogen was evacuated. Three days later the patient died. Gas was not found at the autopsy in the blood or internal organs. The bacillus isolated from the gaseous phlegmon was imperfectly studied, but is identified by Passow with Fraenkel's bacillus of gaseous phlegmon. It caused rapid liquefaction of gelatin which gave a penetrating odour of sulphuretted hydrogen. Clear spaces were seen in the stained bacilli, but it was not determined whether these were spores. Cultures and the wound secretion were not pathogenic for mice and rabbits. Passow's description of his bacillus hardly suffices for its identification, and the strong odour of sulphuretted hydrogen in gelatin cultures is not in favour of its being the bacillus aerogenes capsulatus, if the culture was pure. It is not even clear from Passow's statements that the bacillus was incapable of aerobic growth, or that it was separated in culture from the staphylococci which were also present in the phlegmon.

The preceding observations are all which we have found relating to the bacillus aerogenes capsulatus in the literature since the publication of Welch and Nuttall. There have been reports of cases by Pantzer, Ross, and others of gas in the tissues and serous cavities, in which the opinion has been expressed that this bacillus was concerned, but bacteriological examinations were not made. Reference will be made subsequently to reports of cases of gaseous phlegmon, gas cysts, etc., in which other bacteria than our bacillus were found.

We will now report our personal observations of additional, hitherto unpublished, cases in which the bacillus aerogenes capsulatus was found either during life or at autopsy. Only such details of these cases will be given as are necessary for an understanding of the relations of the bacillus to the case.

The methods of bacteriological examination adopted have been

essentially those described by Welch and Nuttall, and their description need not be repeated in connection with the report of each case. In all cases cover-glass preparations were examined from the original material. Anaerobic cultures were made usually in one-per-cent glucose agar. Occasionally sodium-formate agar was used. We employed, as a rule, the Buchner method, less frequently displacement of the oxygen by hydrogen. Pure cultures were obtained from single colonies, and were planted in various media.

We made continual use of the intravenous inoculation of rabbits, which were killed by a blow at the back of the neck a few minutes after the inoculation. At suitable temperatures the development of the bacilli and of gas in these dead rabbits is rapid and extensive. In this procedure the body of the dead rabbit previously inoculated in the manner described may be regarded as a receptacle containing the best possible nutritive medium and presenting the best conditions for anaerobic growth so far as this bacillus is concerned. By making cultures from these rabbits at a period not too long after the inoculation it was often possible to separate the bacillus from other micro-organisms which might be mixed with it.

Rabbits, guinea-pigs, pigeons, and mice were inoculated subcutaneously and pathogenic effects noted. The results of the experiments upon these animals will be described after consideration of the cases in which the gas bacillus was found. We shall also reserve to a later consideration certain variations observed in the characters of the bacillus and its differentiation from allied anaerobic bacteria.

Unless otherwise stated, the cases were observed in the Johns Hopkins Hospital and Pathological Laboratory. They are arranged as far as possible in groups, but not in the chronological order of their occurrence. On account of their number they are presented in as abbreviated a form as is consistent with clearness. Our autopsies are, as a rule, unusually fresh, and the bodies are kept after death in a large ice chamber. They were made by one of us (Flexner), also by Drs. Barker and Blumer for whose assistance in our studies we desire to express our thanks.

I. Boy, thirteen years old, admitted to the service of Dr. Halsted, March 29th, fourteen hours after gunshot wound of left knee. Examination upon admission showed an ecchymosed bullet wound at the lower outer edge of the patella, with effusion into the knee joint. Flexion and extension gave rise to a peculiar sound, resembling the passage of gas along the intestine. The diagnosis of gas in the joint was made. No emphysema of extra-articular tissues. Temperature, 101·5° F. Twenty-three hours after the injury the joint was freely opened by Dr. Halsted. It contained partly coagulated blood and bloody fluid in which many gas bubbles were present. Tissues about the joint œdematous. A large conical bullet was chiselled from the head of the tibia a short distance below the cartilage. The operation was aseptic throughout, only sterilized instruments coming into contact with the bullet. After the operation the joint was freely irrigated with sublimate solution (1 to 1,000). On account of the persistence of high temperature and the extensive infection of the tissues above and below the joint, the thigh was amputated at the upper third on April 5th. The wound was treated by the open method in the bath, and there was ultimate recovery. Examination of the amputated leg showed extensive soft, reddish-yellow necrosis of the muscles, with separation of the muscles by bloody œdema and sloughy tissue. Gas bubbles were present in various parts of the affected tissues in moderate number. There were some foci of pus, but, in general, the process was one of necrosis with œdema rather than of suppuration.

Cover slips from the blood in the joint at the first operation showed numerous bacilli with the morphology of the gas bacillus and a smaller number of cocci. Anaerobic and aerobic agar cultures were made both from this bloody fluid and from the bullet, which, seized by sterile forceps, was immediately dropped into a tube of high melted agar. Numerous colonies of the bacillus aerogenes capsulatus developed in the anaerobic cultures, most rapidly and abundantly from the one inoculated with the bullet. In smaller number there grew colonies of the staphylococcus aureus and the streptococcus pyogenes. Cultures and cover slips from the second operation still showed the gas bacillus, but now the streptococci predominated. Typical gas development in blood and organs followed intravenous inoculation of rabbits which were killed soon after the inoculation. The bacillus caused the death of pigeons in seven to twenty-four hours, with the characteristic lesions. Gaseous phlegmon followed subcutaneous inoculation of guinea-pigs.

The notable features of this case are the infection of the joint by a bullet carrying the gas bacillus, the rapid development of gas in the joint, so that its presence could be recognised by manipulation fourteen hours after the injury and before the joint was opened by the surgeon, the rapid extension of the necrotic and gaseous inflammation to the surrounding tissues, and the ultimate recovery after amputation.

II. November 9th, man admitted to the service of Dr. Halsted with the history that his left leg had been run over by a car and shortly before admission had been amputated at about the junction of the middle and lower third of the leg. On removing the dressings, which were saturated with blood, there was so much bleeding from the stump that an Esmarch bandage was applied above the knee. The skin was covered with dirt and sand, and showed no evidence of having been cleansed before the amputation. The wound was opened and irrigated, and after ligation of the vessels it was partly closed and drained with gauze. At this time there was no emphysema of the tissues. Twenty-four hours later the temperature rose with a chill to 104° F., the pulse ranged from 120 to 160, and there was much pain and tenderness in the leg. Extending from the edges of the wound nearly to the patella, the skin, which was not much reddened, was the seat of emphysematous crackling over the anterior and lateral aspects of the leg. Free incisions were made and gas was found in the subcutaneous and intermuscular tissues with extravasated blood and bloody turbid exudate. Although temporarily relieved, the condition of the patient became critical, and amputation was done at the lower third of the thigh by Dr. Bloodgood. After this the patient made a good recovery.

Cover slips from the blood-stained fluid, which escaped at the first incision into the emphysematous tissues, contained numerous bacilli with the morphology of the gas bacillus, many being contained within leucocytes with polymorphous nuclei, and many also being free. Chains and clumps of cocci were also seen. Cultures gave the bacillus aerogenes capsulatus with typical reactions and, in addition, the staphylococcus albus.

This case belongs also to the group of gaseous phlegmons, infection with the gas bacillus being probably referable to the dirt which was ground into the leg by the passage over it of a car wheel. As in the preceding case, the gas bacillus was associated with pyogenic cocci. Here also recovery followed amputation, the previous treatment by free incisions not having held the infectious process in check.

III. June 2d, man admitted to the service of Dr. Halsted with the history that eight days previously he had fallen from a wagon and injured his right hip and thigh. On admission, temperature 101° F., subsequently rising to 104° ; pulse, 80 to 90. Thigh flexed on the pelvis, the leg on the thigh, the limb being abducted and rotated outward. No external wound of integument. The thigh, especially on the inner and posterior aspects, much swollen and tender, with emphysematous crackling on the inner side and in the popliteal space. The skin over these parts is reddened. A similar condition in the gluteal region. By rectal examination a tender swelling can be felt about four centimetres above the anus. An incision was made from the tuberosity of the ischium to the popliteal space. The subcutaneous tissues were infiltrated with serum. The deep fascia was

tense and thickened by a fibrinous exudate. Upon incising this tense fascia a turbid grayish fluid mixed with gas bubbles and having a fæcal odour escaped. The muscles and tissues were much disintegrated. As the process extended down the leg, incisions were continued to the heel, the whole lower extremity being red, swollen, and emphysematous.

Death on June 7th. Autopsy twelve hours after death. The tissues of the gluteal region and right lower extremity extensively necrotic and infiltrated with offensive, dirty, turbid, grayish-red exudate. The muscles are friable and disintegrated. In places there is pus. Gas bubbles are present, most abundantly in the gluteal region. The necrotic inflammation which has dissected out the sciatic nerve can be followed through the sciatic notch into the pelvis, where there is a perirectal phlegmon the size of an orange pushing up the peritonæum, which is greenish in colour over the prominence but not inflamed. The perirectal abscess communicates by two perforations with the rectum. Moderately large probes can be passed from the rectum through these openings. Peritonæum smooth, without excess of fluid. No gas in the heart, vessels, or viscera. No fracture of the pelvis.

Cover slips from the gas-containing fluid which escaped at the incision into the thigh during life showed a large number of bacilli with the morphology of the gas bacillus. This was the predominant organism, but in addition were seen a short, oval bacillus, slender bacilli, and cocci in long and short chains, the picture being suggestive of that of intestinal bacteria. The cultures gave abundant colonies of the bacillus aerogenes capsulatus, which was tested upon animals, and also streptococci. The same bacteria were found by cover slips and cultures at autopsy in the necrotic inflamed tissues. Aerobic and anærobic cultures from the blood and organs were sterile except that the gas bacillus was isolated in anærobic culture from the congested lower lobes of the lungs, where it was also demonstrated by direct microscopical examination.

In this case a most extensive fatal emphysematous cellulitis followed traumatic rupture of the rectum, the process extending rapidly from the ischio-rectal tissues through the sciatic notch into the gluteal region and down the thigh, nearly the whole lower extremity becoming involved. It is clear that the bacillus aerogenes capsulatus in this case escaped with other bacteria from the intestine through the rectal perforations, and grew during life abundantly in the tissues.

IV. For the following unpublished case we are indebted to Dr. Tiffany, Professor of Surgery in the University of Maryland, who has kindly furnished us the history. A vigorous telegraph lineman, twenty-seven years old, at 10.30 A. M. fell from a telegraph pole, striking his right knee against the curbstone, which produced a compound comminuted fracture of the

patella. The trousers were not torn through, but the underclothing was ground into the wound. He was seen an hour and a half after the injury by Dr. Tiffany, who opened the joint freely, removed the small fragments of patella, and stitched the fibrous capsule. The patient that night became incoherent. The next morning the temperature was 101·5° F., the pulse 118. Delirium occurred in the afternoon. The temperature continued to rise, the pulse became more and more rapid and thready, and the next morning the patient died, less than forty-eight hours after the injury. Gas was detected by the resident physician in the tissues a short distance from the wound a few hours before death, and Dr. Tiffany, within two minutes after death, found by palpation emphysematous crackling in the tissues three inches above the site of the patella, and also in a dusky area over the tibia five inches below the patella. The tissues in these situations were somewhat swollen and œdematous. Upon incision a reddish serous exudate containing gas bubbles escaped. There was no pus. An autopsy was not permitted.

Cultures made by Dr. Mann gave the bacillus aerogenes capsulatus with its characteristic reactions. No other organism developed in the anaerobic cultures, and aerobic agar plate cultures were sterile.

This case of emphysematous cellulitis is notable for its extremely rapid and fatal course, death occurring within less than forty-eight hours after the injury. As the outer clothing was not torn, it seems probable that the gas bacillus entered through bits of cloth from the drawers having been ground into the wound. The infection was apparently due exclusively to the gas bacillus.

V. For the following case we are likewise indebted to Dr. Tiffany. At 12.30 A. M. the right forearm of a healthy railway brakeman was crushed between the buffers of two cars and the clothing was ground into the wound. The wound was dressed by two physicians near the scene of accident, and the patient was transferred by train a distance of forty-three miles to Baltimore, where he was seen in the hospital about ten hours after the injury by Dr. Tiffany, who tried to save the limb, which was much mangled. The following morning, thirty-three hours after the injury, emphysematous crackling was noticed about the wrist. The tissues were becoming œdematous. The skin over the doughy crepitating swelling was pale. Two hours later amputation midway between the elbow and shoulder was performed. The ulnar and radial arteries were destroyed. The patient made a good recovery.

Cover-slip preparations and cultures were made both before the operation and immediately after amputation. The cover slips showed bacilli like the gas bacillus and streptococci. Cultures yielded the bacillus aerogenes capsulatus and pyogenic cocci. There was also a spore-producing anaerobe which appeared with the gas bacillus in pigeons and guinea-pigs inoculated with the cultures of the first generation, but which did not appear in the

subsequent cultures and did not grow in the sugar agar cultures made from these animals. The gas bacillus gave characteristic reactions in cultures and in inoculated animals.

VI. Dr. Mann has kindly furnished the notes of the following case. Woman, seventy-four years old. When first seen by Dr. Mann on January 18th the hand, forearm, and arm were much swollen, with red lines extending upward from the elbow. The hand was gangrenous, green, with blebs containing bloody offensive fluid. Emphysematous crackling could be felt over the whole extremity as far as to the deltoid muscle. The general condition of the patient was bad, there being high fever, rapid pulse, and delirium. The arm was amputated near the insertion of the deltoid. Examination of the amputated arm showed that the tissues were disorganized and infiltrated with offensive bloody exudate. Gas bubbles were present throughout the tissues and exudate. The patient's general condition was improved the day after the amputation, and the crackling disappeared in the tissues above the line of amputation. Four days after the operation symptoms of tetanus appeared, and death occurred from tetanus on January 24th. No autopsy was obtained.

Cultures made from the amputated arm gave streptococci and the bacillus aerogenes capsulatus, which was fully identified. Cultures made from the wound after amputation did not show the gas bacillus.

The preceding six cases belong to the category of gaseous phlegmons, emphysematous cellulitis, or emphysematous gangrene. All were severe infections, three terminating in recovery after amputation and three ending fatally. In only one of the fatal cases was an autopsy performed, and in this gas was not present in the blood and internal organs, although the gas bacillus was cultivated from the lung in Case III.

The following case is inserted in this connection as there was severe phlegmonous inflammation, although gas and the gas bacillus were found only at the autopsy:

VII. Woman, admitted to the service of Dr. Halsted June 24th with phlegmonous infection of the hand and forearm, the joints of the wrist, hand, and fingers being involved. Fever and rapid pulse. June 25th, free incisions into the hand and forearm with sublimate irrigation. The sheaths of the anterior tendons of the fingers contained pus. June 27th, general condition bad; no tendency to wound repair. Offensive odour of the discharges. No emphysema detected. Amputation at the middle of upper arm. Tissues œdematous and vessels thrombosed. Patient died June 30th.

Bacteriological examination of the phlegmon and wound during life showed streptococci and staphylococci. No gas bacilli were detected nor emphysema of the tissues. Cultures from the blood during life were sterile.

At the autopsy, twenty-four hours after death, the spleen was large and soft, the kidneys large, smooth, and pale, being the seat of chronic diffuse nephritis. Sticky purulent discharge covered the exposed surface of the amputation wound. Gas was present in moderate amount in the blood of the heart and vessels; none in the parenchyma of the organs.

Cover slips from the heart's blood showed many bacilli resembling the gas bacillus. Aerobic cultures from the blood, liver, kidney, and lungs were crowded with colonies of the bacillus coli communis. Anaerobic cultures from the same situations and from the spleen gave numerous colonies of the bacillus aerogenes capsulatus with typical reactions. A pigeon inoculated subcutaneously and into the breast muscle with one cubic centimetre of the heart's blood of a rabbit containing numerous bacilli developed after a few hours an emphysematous swelling, but subsequently recovered.

As the presence of the gas bacillus was not determined during life, its development in the blood with the formation of gas is probably to be regarded as occurring after death. No positive statement can be made as to whether the invasion of the bacillus was through the wound.

We pass now to our observations of a group of cases of perforative peritonitis in which the bacillus aerogenes capsulatus was found.

VIII. Woman, admitted to the service of Dr. Osler February 1st with history of pain in the region of the stomach, vomiting, and emaciation extending over three months and increasing in severity during last three weeks. On day of admission she was suddenly seized with a sharp paroxysm of intense pain localized over the stomach. Vomited reddish fluid containing red blood-corpuscles. The abdomen became distended, tympanitic, and tender on pressure. The patient passed into a state of collapse and died at 5.20 P. M. on the day following admission to the hospital.

Autopsy fourteen hours after death. The abdomen is greatly distended and tympanitic, and upon incision there is a free escape of gas from the peritoneal cavity. There is a thin fibrinous exudate over the general peritoneal surfaces. The cavity contains about 2,000 cubic centimetres of thin, grayish, turbid fluid holding flakes of fibrin. An infarcted multilocular ovarian cystoma with twisted pedicle nearly fills the pelvic cavity. The intestines are markedly distended with gas. There are multiple necroses in the fat about the pancreas and in the omentum and mesentery. One and a half centimetre below the pylorus there is an ulceration with thickened indurated walls, and 12 millimetres below this a second similar ulceration through all of the coats of the duodenum. The floor of one is completely closed by adhesion to the pancreas, but that of the other is only partly closed by adhesions, so that there exists here a perforation 5 millimetres in diameter leading into the general peritoneal cavity. Microscopical examination of the thickened edges of the duodenal ulcers showed them to be carcinomatous. The gastro-hepatic lymphatic glands

are the seat of cancerous infiltration. No metastases in the liver. The rootlets of the portal vein contain small gas bubbles, but no gas bubbles are found in the vessels elsewhere, in the liver or in other parts than those mentioned.

Cover-slip preparations from the fibrinous exudate in the peritoneal cavity showed many bacilli with the morphology of the bacillus aerogenes capsulatus. No other bacterial species was found in these preparations. Polynuclear leucocytes were present in only moderate number. Anaerobic cultures from the fibrinous exudate covering the peritoneal surface of the liver gave numerous colonies of the gas bacillus with all of the characteristic reactions. No other species developed. Cultures from the heart's blood sterile. The bacillus isolated from this case was used for numerous experiments on rabbits, pigeons, and guinea-pigs. In rabbits subcutaneous inoculation produced gaseous œdema with recovery. For pigeons and guinea-pigs the bacillus was highly pathogenic with typical lesions, including the production of peritonitis by intraperitoneal inoculation.

This case of fibrino-purulent pneumoperitonitis following perforation of a cancerous ulcer of the duodenum is of especial interest from the fact that at the autopsy fourteen hours after death, the body having been kept in an ice box, the bacillus aerogenes capsulatus was present in large number, and apparently in pure culture in the exudate. Other bacteria, if present at all, were in such small number that they were not detected either in cover-slip preparations or in aerobic or anaerobic cultures. We have occasionally observed in other cases of perforative peritonitis the predominant or apparently exclusive presence of a single bacterial species. Gas bubbles were absent from the blood with the exception of the rootlets of the portal vein. The peritoneal exudate was fibrino-serous without many pus cells.

IX. Service of Dr. Osler. Man, forty-four years old, died July 30th from typhoid fever, on the eighteenth day of the disease, with symptoms of perforative peritonitis. Autopsy five hours after death. The abdomen is greatly distended and very tense. Upon incision there is free escape of gas from the peritoneal cavity. Intestines tympanitic. There is diffuse fibrinous exudate over the visceral and parietal peritonæum with a moderate amount of fluid, which in some places is pus, in other places turbid serum. Thirty-nine centimetres above the valve is a circular perforation, 3 millimetres in diameter, of a typhoid ulcer. There are many ulcers of the ileum, for the most part clean, and the other usual lesions of typhoid fever. There is acute hæmorrhagic nephritis and many small abscesses are scattered through the kidney containing typhoid bacilli. Gas bubbles are not present in the blood vessels and viscera.

Cover slips of the peritoneal exudate show various bacteria, including streptococci, bacilli with the morphology of the colon bacillus, slender bacilli, and plump, capsulated bacilli resembling the bacillus aerogenes capsulatus. The last species was present in large numbers. The bacillus aerogenes capsulatus with other species was obtained from the peritoneal exudate, and from the organs the bacillus typhosus (in the bile, bone marrow, mesenteric glands, and kidneys) and the bacillus coli communis grew in the cultures.

X. Service of Dr. Osler. Man, died September 9th from typhoid fever in the third week of the disease. Twenty-four hours before death there was severe abdominal pain, followed by symptoms of perforative peritonitis.

Autopsy eighteen hours after death. Abdomen much distended and drumlike. Upon incision of the peritoneal cavity there is violent escape of gas. There is diffuse fibrinous peritoneal exudate. The cavity contains about 1,000 cubic centimetres of turbid fluid mixed with fæcal material. There are two small perforations of typhoid ulcers of the ileum. There are many typhoid ulcers, some with necrotic base. The spleen is large and soft. Focal necroses are in the liver. No gas in the heart or viscera.

Microscopical examination of the peritoneal exudate shows the presence of comparatively few cells, but an enormous number of bacteria of various kinds. The bacillus aerogenes capsulatus was obtained from the exudate, being present in fair numbers, but the predominating organism was the colon bacillus. There were a few streptococci.

XI. Service of Dr. Osler. Man, died November 26th from typhoid fever, in the fourth week, with symptoms of perforative peritonitis, which developed two days before death.

Autopsy twenty-two hours after death. Abdomen distended, tympanitic, and with beginning greenish discoloration. Gas escaped abundantly on opening the peritoneal cavity. There is extensive fibrino-purulent exudation, most abundant in the lower half of the peritoneal cavity. There is considerable brownish-yellow fluid with fæcal odour. The serosa is intensely hyperæmic. There are two pin-hole perforations of typhoid ulcers in the vermiform appendix, which lies buried in a mass of fibrinous exudate. There are several typhoid ulcers in the small and large intestine. Gas bubbles are present in the inferior vena cava, the systemic and portal veins, and in the right heart.

Cover slips and cultures from the peritoneal exudate gave the bacillus aerogenes capsulatus, the bacillus coli communis, and streptococci. The gas bacillus predominated. This same bacillus was found in large number and pure culture in the blood, with the exception of one colony of the colon bacillus in a gelatin roll from the heart's blood. Aerobic cultures from the spleen and liver were sterile.

The three last cases are all examples of pneumoperitonitis due to perforation of typhoid ulcers. The bacillus aerogenes capsulatus was mixed with various intestinal bacteria in the exudate, and in

such numbers as to indicate its multiplication in the peritoneal cavity, although to what extent this occurred during life can not be determined. Inasmuch as we have evidence that this bacillus is capable of causing peritonitis, with formation of gas, its presence under these circumstances is not a matter of indifference, even when associated with such recognised pathogenic species as the pyogenic cocci and the colon bacillus. The amount of gas in cases of peritonitis due to intestinal perforation is extremely variable, and it is noticeable that in the preceding cases it was in large quantity in the peritoneal cavity, and that the intestines were markedly tympanitic. The gas found in the heart and vessels in the last case was doubtless referable to post-mortem development of the gas bacillus, the autopsy being twenty-two hours after death with evidences of beginning cadaveric decomposition.

XII. Service of Dr. Kelly. Negress, aged thirty-nine, admitted June 26th with strangulated umbilical hernia and fæcal vomiting. The strangulation had existed for two days before admission. Temperature 102° F. General condition bad. Herniotomy was performed. Strangulated omentum was excised and the gangrenous gut stitched to the abdominal wall. Death after forty-eight hours.

Autopsy twelve hours after death. Gas in the peritoneal cavity, which is shut off from the artificial anus formed at the umbilicus, this anus being in a loop of intestine 185 centimetres above the valve. Visceral and parietal peritonæum hyperæmic and coated with a thin layer of fibrin. The intestinal loops are adherent to each other and to the omentum by fresh fibrin. Old fibrous adhesions in pelvic peritonæum and over liver and spleen. General arterio-sclerosis. Several small old ulcers in the cæcum.

Cover slips from the fibrinous exudate on the peritonæum show as the predominating organism the bacillus aerogenes capsulatus. In addition a few smaller bacilli and cocci are seen. Cultures furnished the bacillus aerogenes capsulatus and also the bacillus coli communis and a few streptococcus colonies. Cultures from the blood negative; from the kidney, liver, and spleen, the colon bacillus.

In this case the gas bacillus doubtless entered the peritoneal cavity from the strangulated gut, but whether or not before the operation was not determined. The exudate was fibrinous, not purulent. The predominance of the gas bacillus in the exudate at the fresh autopsy indicates that it was a factor in the production of the peritonitis.

The last five cases, together with Case III, afford conclusive proof that the bacillus aerogenes capsulatus is sometimes an inhabitant of the intestinal canal, from which it may invade other parts of the body. That it may occur also in the alimentary canal of animals is proved by the following observation:

On the morning of December 10th one of the laboratory stock of rabbits which had not been used for experiment refused to eat and remained quiet in its cage. It was found dead in the afternoon at three o'clock. It was alive an hour before. The body was still warm and not stiff. The rabbit was placed on ice and the autopsy made two hours later. The peritoneal cavity was greatly distended with gas. It contained some blood-stained fluid which had accumulated, especially about the omentum, which was of a deep-red colour. The stomach was moderately distended with gas, and in its anterior wall was a perforation 2 millimetres in size, through which food extruded. On opening the stomach there were found, in addition to the perforated ulcer, two other circular ulcers, with red edges, one 3 millimetres and the other 4 millimetres in diameter, extending into the submucosa. The intestines were not especially tympanitic. There was no pus in the peritoneal sac. No other lesions were found. There was no gas in the vessels or viscera.

Cover slips from the bloody fluid in the peritonæum showed in large numbers, bacilli with the morphology of the gas bacillus and here and there a coccus. Anaerobic cultures from the fluid yielded numerous colonies of the bacillus aerogenes capsulatus with typical reactions and a few colonies of a white staphylococcus.

The occurrence of perforating gastric ulcer as a spontaneous disease in the rabbit is of much interest. The ulcers seemed to be of hæmorrhagic origin. The presence at the autopsy, made shortly after death, of a hæmorrhagic condition of the omentum and of bloody fluid and of gas in large amount in the peritoneal cavity indicates that the perforation occurred during life. No other cause of death was found. Under these circumstances the large number of gas bacilli, predominating greatly over the other bacteria, in the peritonæum is significant. There was no trace of the post-portem digestion of the stomach sometimes found in rabbits.

The following case presents many points of interest, especially the evidence of the production by the gas bacillus of pneumoperitonitis without perforation, of gas in diabetic urine, and of gas cysts in the intestine:

XIII. Service of Dr. Osler. Man with diabetes mellitus and cirrhosis of the liver. Had been ill for nearly two years. Had several profuse hæmorrhages from the stomach during the six months preceding death. Developed thrombosis of right femoral vein three months before death. Abdomen became distended and tender. A week before death severe abdominal pain, most marked in right hypochondrium, set in and the abdomen became very much distended. There was a uniform tympanitic sound over the anterior surface of the abdomen. The region of liver dulness was tympanitic. The patient died comatose April 6th. Peritonitis from perforation of a gastric or duodenal ulcer was the clinical diagnosis. Autopsy was made six hours after death. No trace of cadaveric decomposition. Considerable subcutaneous emphysema, especially in the neck and thighs. Heart and blood vessels contain gas bubbles. On opening the distended and tympanitic peritoneal cavity a large amount of gas, free from foul odour, escapes. Intestines moderately distended with gas. Considerable amount of blood-stained turbid serous fluid in the peritoneal sac. No actual pus. Visceral peritonæum hyperæmic. There is a general fibrinous exudate, in most places thin, but thicker over hæmorrhagic areas in the intestine. There is hæmorrhagic infarction of the jejunum in three places, the largest being twenty-five centimetres in length. Here the coats of the intestine are firm, thickened, and of a uniform reddish-black colour, from infiltration with extravasated blood. There is no perforation of the intestine or stomach. The mesenteric veins are extensively thrombosed, likewise the splenic and portal. There is a collection of cystic cavities filled with gas extending over an area ten centimetres long in the jejunum, which is here not infarcted. These cysts form projections into the lumen of the intestine, some being as large as a bean. They lie in the submucous and mucous membranes. Similar gas cysts are present in the mesentery near its attachment to the intestine. Gas bubbles are present in the omentum. The capsule of the liver is much thickened by diffuse fibrous growth and there is cirrhosis. The urinary bladder is distended with pale, frothy urine due to mixture with gas bubbles. Gas holes are not present in the liver and solid organs, with the exception of the mesenteric glands, which contain small gas vesicles. The endocardium, particularly of the right side, shows diffuse imbibition of blood-colouring matter.

Cover slips of the blood show bacilli with the morphology of the gas bacillus. Those from the peritonæum show the gas bacillus in large numbers; in much smaller numbers also bacilli resembling the colon bacillus. Gas bacilli are in apparently pure culture in the various thrombi in the abdominal veins. They are found mixed with other bacteria in the intestine.

Aerobic plate cultures from the heart's blood, lungs, spleen, kidneys, peritonæum, and liver gave colonies of the colon bacillus. Anaerobic plates from the blood, peritonæum, and urine gave numerous colonies of the bacillus aerogenes capsulatus with typical reactions. From the intestine the gas bacillus and the colon bacillus were cultivated.

Microscopical examination of sections of the intestine showed extensive invasion by the gas bacillus of the coats in the infarcted areas, where the

mucous and submucous coats were necrotic and filled with fibrin, red and white blood-corpuscles. Sections through intestine which is not the seat of infarction showed dense masses of gas bacilli upon the surface of the mucosa.

The gas cysts of the intestine occupy both the mucosa and the submucosa. Those which begin in the mucosa and remain there lie next to the muscularis mucosæ. Others beginning in the submucosa may involve the mucosa, which is sometimes spread out so as to form a thin film over the surface. The part of the intestine containing these cysts stains poorly, and contains numerous gas bacilli.

In the preceding case a man with diabetes and cirrhosis, who had repeatedly vomited blood, developed extensive thrombosis of the mesenteric, splenic, and portal veins, leading to hæmorrhagic infarction of the intestine. During the last few days of life the symptoms of perforative peritonitis manifested themselves, but the autopsy, made only six hours after death, showed that there was no perforation of the stomach or intestine, although the inflamed peritoneal cavity contained much gas. Gas had begun to make its appearance in the blood vessels, and there was subcutaneous emphysema. Gas cysts were in the intestinal wall, and there was pneumaturia. The bacillus aerogenes capsulatus had invaded the peritoneal cavity evidently through the infarcted wall of the intestine. In consideration of the symptoms of pneumoperitonitis during life, of the short time which had elapsed between the time of death and the autopsy, of the conditions found at autopsy, and of the experimental results from inoculation of the bacillus, it seems to us altogether probable, if not absolutely demonstrated, that in this instance the gas bacillus was the cause of pneumoperitonitis without the existence of any communication between the peritoneal cavity and an air-containing viscus or the exterior. It is true that the bacillus coli communis was also present, although in smaller number than the bacillus aerogenes capsulatus, and that the colon bacillus has been claimed, especially in cases of diabetes, as a cause of pneumaturia and gaseous phlegmon, but whatever its status as regards these affections may be, there are no observations which show that the colon bacillus can produce general subcutaneous emphysema, gas bubbles in the blood vessels, or gas in the serous cavities, whereas we have

positive evidence of the capacity of the bacillus aerogenes capsulatus to cause such conditions as were present in this case. Diabetes mellitus is doubtless a factor favourable to the fermentative production of gas, especially in the urine. Pneumaturia was not observed during life, but it is a symptom which can be readily overlooked when the gas is present in the urine in small amount, and it was not especially looked for.

In the following case the gas bacillus was also associated with non-perforative acute peritonitis, but the evidence as to its causative relation to the peritonitis is not conclusive:

XIV. Service of Dr. Kelly. Negress, sixty years old, with arteriosclerosis, chronic diffuse nephritis, anasarca, and ascites. During last two weeks of life she vomited almost incessantly and developed symptoms of acute peritonitis. Died July 5th.

Autopsy twenty-one hours after death; no greenish discoloration; about 4,000 cubic centimetres of cloudy yellowish-red fluid in peritoneal cavity. Visceral peritonæum congested and covered by a thin layer of fibrin. Gas was not noticed on opening the abdominal cavity, but it might have escaped observation. All of the cavities of the heart contain gas bubbles, which are present also in the blood vessels. No subcutaneous emphysema or gas holes in the organs. Diffuse red staining of endocardium and intima. Small contracted kidneys. Sclerosis of the arteries. Old fibrous adhesions about the lungs, liver, spleen, and pelvic viscera. Intestines normal save swelling of the lymphatic follicles.

Cover slips from the heart's blood and peritoneal cavity showed numerous bacilli with the morphology of the gas bacillus which was obtained in culture from these situations and when inoculated into pigeons produced characteristic gaseous phlegmon. Aerobic cultures yielded many colonies of the colon bacillus from the peritonæum and lungs and a few from the spleen, liver, and kidneys.

In the preceding case the possibility that the peritoneal cavity was invaded by the gas bacillus after death can not be denied. Doubtless the formation of gas bubbles in the heart and vessels was post mortem. As the presence of gas in the peritoneal cavity was not suspected, no especial attention was directed toward its detection. A moderate amount of gas in the peritoneal sac may be readily overlooked, as it escapes on free incision. The peritonitis was characterized by a thin fibrinous exudate, turbid blood-stained fluid, and redness of the visceral peritonæum, these being the char-

acters which we have found associated with the presence of the bacillus aerogenes capsulatus under conditions indicating the ante-mortem invasion of this organism. The peritonitis was associated with chronic Bright's disease.

In the preceding fifteen observations (including that of the rabbit) the bacillus aerogenes capsulatus invaded the body apparently either directly from without or from the stomach or intestine. In the two following cases it is probable that the bacillus entered by way of the lungs.

XV. Service of Dr. Osler. Woman with acute and chronic valvular endocarditis, chronic Bright's disease, ascites, and pleurisy. Died September 4th. Autopsy twelve hours after death. Peritoneal cavity contains several thousand cubic centimetres of clear straw-coloured serum. Peritonæum smooth. Left pleural cavity contains several hundred cubic centimetres of blood-stained serum. Visceral pleura coated with shaggy fibrin. Many subpleural ecchymoses. Left upper lobe and upper part of lower lobe compressed. Remainder of lower lobe solidified by extensive hæmorrhagic infarction. The branch of the pulmonary artery supplying left lower lobe occluded by firm reddish-gray thrombus, with central softening. Chronic and acute vegetative endocarditis of mitral valve. Hypertrophy and dilatation of heart with ante-mortem thrombi in right and left auricular appendages. Red, granular kidneys. No subcutaneous emphysema or gas bubbles in the heart, blood vessels, or organs.

Cover slips from the exudate in pleural cavity shows capsulated diplococci and also many encapsulated bacilli with the morphology of the bacillus aerogenes capsulatus, which was isolated in culture. The same bacillus was found in the infarcted area in the lung and in small numbers in the bronchial mucus. Aerobic and anaerobic cultures from the blood and other organs were sterile.

The presence of gas bacilli in this case at fresh autopsy in the infarcted lung and pleural cavity, and their absence from the blood vessels and other organs, indicate that this bacillus invaded the pleural cavity from the lung. The consolidation of the lung by hæmorrhagic infarction undoubtedly favoured the lodgment and survival of the bacillus in this situation. As the presence of gas in the pleural cavity was not suspected, the chest was freely opened at autopsy without taking any precaution to determine its presence. The lanceolate coccus was probably the primary cause of the pleurisy, but in view of Levy's observation, already cited, of the gas

bacillus in a case of pneumothorax without perforation, and of our Case XIII of pneumoperitonæum due to the gas bacillus without perforation, it is permissible to suppose that in the present case the gas bacillus may have multiplied in the pleural cavity and produced gas during life, although we can furnish no positive proof of this from the autopsy, and confirmatory evidence from examination of the chest during life is lacking.

XVI. Service of Dr. Osler. Man with thoracic aneurism, aortic insufficiency, and chronic passive congestion with pulmonary infarctions. Died February 14th. Autopsy twenty-four hours after death. Œdema of extremities. Bloody, frothy fluid oozes from nose and mouth. Intestines distended with gas. Peritonæum smooth. In right pleural cavity considerable quantity of blood-stained turbid fluid. Fresh fibrinous exudate over visceral pleura, which is ecchymosed. There are numerous hæmorrhagic infarctions in right middle and lower lobes, which contain very little air and a few scattered wedge-shaped infarctions in the upper lobe. The infarctions have a peculiar opaque colour and contain gas. The whole lung is congested, and contains abundant frothy, bloody fluid. Branches of the pulmonary artery contain many ante-mortem thrombi. The left lung likewise contains scattered hæmorrhagic infarctions, and presents a condition similar to that of the right. The corresponding pleura is coated with fibrin, but there is little fluid in the cavity. Heart is hypertrophied and dilated; aortic valve insufficient from chronic thickening; marantic thrombi in right auricular appendix and in apex of right ventricle. Aneurism about the size of a small orange involving ascending and part of transverse aortic arch. Atheroma of aorta. Moderate atrophic cirrhosis of liver. No abnormality of stomach or intestine.

The endocardium of the right heart and the intima of the pulmonary vessels and of the veins adjacent to heart are diffusely reddened. A similar reddening does not exist in the left endocardium or the systemic arteries. Gas bubbles are present in the pulmonary vessels, the right heart, the venæ cavæ, the jugular, femoral, and portal veins. They are absent from the left heart, the systemic arteries, and the abdominal viscera. No subcutaneous emphysema.

Cover slips from the blood in the right heart and the adjacent veins show many bacilli indistinguishable from the gas bacillus, and apparently unmixed with other bacteria. A surprisingly large number of the same bacilli, likewise without other bacteria, are seen in cover slips from infarcted areas in the lung. Cultures from the blood of the right heart, thrombi, infarcted lung, and veins containing gas furnished abundant colonies of the bacillus aerogenes capsulatus. No other organism was found except streptococci in an anaerobic culture from the heart's blood. Aerobic cultures from blood and organs were sterile. As in other cases, animals were inoculated with the cultures of the gas bacillus which caused the

characteristic lesions in guinea-pigs and pigeons, and gave the typical reaction when inoculated into the circulation of rabbits which were killed shortly afterward.

Microscopical examination of sections of the lung showed extensive desquamative pneumonia and older and fresh infarctions. The gas bacilli were demonstrated in large numbers in the larger pulmonary vessels and capillaries, in the lymphatics and in the alveoli of the lung. There was considerable infiltration of the margins of the infarctions with polynuclear leucocytes, which often filled up the lymphatics.

It may be mentioned that an autopsy was performed immediately after this one upon a man dying of phthisis and croupous pneumonia within an hour of the death of this patient, and that no gas or gas bacilli were found, so that the latter autopsy may serve as a control. There were no putrefactive changes in either case, the bodies having been kept on ice.

Inasmuch as in the preceding case gas bacilli were found in very large numbers in the pulmonary infarctions, more abundantly than elsewhere, and as gas bubbles were present only in the right heart and the vessels leading to and from this side of the heart, we consider it probable that the invasion of the bacillus in this case took place from the lungs. Invasion from the intestine can not be positively excluded, but if this had been the direction we should have expected to find development of gas in the liver. Welch and Nuttall demonstrated in their experiments that gas bacilli introduced after death into the right ventricle develop with formation of gas in the course of the vessels leading to and from this side of the heart, and that only after a certain time do they appear in the systemic arteries and the organs. We think it likely, from the conditions found at autopsy, that the bacilli entered the infarcted lung during life, but to what extent they may have multiplied during life we can not say. Nor can any positive statement be made as to whether the bacilli were conveyed to the lung from without through the air passages or were carried there by the circulation. Inasmuch as the gas bacillus is a not uncommon inhabitant of the intestinal canal, it seems to us probable that, like other intestinal bacteria, this organism may, under certain conditions, enter the circulation without doing any harm. Assuming such an invasion in the present case, it would not be surprising if the bacillus should find in infarcted areas of the lung conditions permitting its survival which did not exist

elsewhere. Infarcted areas of the lung are the seat not only of a dense hæmorrhagic infiltration, but of actual necrosis of the lung tissue. That the conditions in infarcted lung tissue are sufficiently anaerobic to permit the growth of the gas bacillus can scarcely be doubted when one considers the dark reddish-black colour of the extravasated blood in pulmonary infarctions. In Case III the gas bacillus seems also to have been localized in the lung, as it was found only there and in the gaseous phlegmon.

In the three following cases the bacillus aerogenes capsulatus was found in inflamed parts of the urinary tract:

XVII. Service of Dr. Halsted. Man, admitted December 25th with retention of urine due to urethral stricture following gonorrhœa. During six weeks preceding admission bladder had been catheterized three times daily. General condition septic. Fever, rapid pulse, tongue dry and coated. Urine contained albumin, casts, and pus. Perineal section performed two days after admission. Vesical symptoms relieved, but general condition remained critical, and patient died January 1st.

Autopsy by Dr. Blumer three quarters of an hour after death in cold weather. Gaping wound in perinæum, communicating with bladder, without signs of repair. Chronic purulent cystitis. Double pyo-ureter and pyonephrosis. Multiple miliary abscesses in the kidneys and the prostate. Stricture of urethra. Chronic interstitial orchitis. Chronic diffuse nephritis. Arterio-sclerosis. Multiple fat necroses in peripancreatic fat, omentum, and panniculus adiposus. Parenchymatous degeneration of heart and liver. A few gas bubbles in the left auricle detected upon first opening the chest.

Cover slips from renal abscesses, renal pelvis, ureters, bladder, and perineal wound showed a large number of bacilli with the morphology of the gas bacillus; also bacilli resembling the colon bacillus, streptococci, and a few rather long, thin bacilli. Contrary to our custom, cultures were not made at the time of the autopsy, but the organs, wrapped in wet cloths, were put on ice. Culture tubes inoculated the following day did not show the gas bacillus. The demonstration of the gas bacillus in this case, therefore, was not complete, and therefore not satisfactory.

In the following case Dr. Blumer cultivated the gas bacillus from the kidney at the autopsy, and the following day failed to obtain it in culture from the same organ, which had remained during twenty-four hours wrapped in wet cloths on ice.

XVIII. Service of Dr. Halsted. Man, with hypertrophied prostate, admitted December 6th with retention of urine and constant dribbling. Patient was in a stupid condition and no history could be obtained. Died comatose January 1st. Only a partial autopsy was permitted. Hypertrophy of prostate. Double pyo-ureter and pyonephrosis. Multiple miliary abscesses in the kidneys. The renal pelves and ureters were distended with gas and

contained pus mixed with bubbles of gas, and small gas cysts were present in the mucous membrane of the pelves, which in places is hæmorrhagic and coated with fibrinous exudate. Chronic diffuse nephritis. Spleen large and soft. Gas not present in the blood and organs other than the urinary.

Cover slips from the kidney, renal pelvis, ureters, and bladder showed as the predominant organism bacilli, sometimes encapsulated, with the morphological characters of the gas bacillus. In addition, bacilli resembling the colon bacillus, thin bacilli, and cocci, in pairs and groups, were present in the same situations. The bacillus aerogenes capsulatus, with its typical reactions, was isolated in culture from the kidneys and urinary passages.

XIX. For the following case we are indebted to Dr. Williams, of Buffalo, who will publish the report in full in the *Bulletin of the Johns Hopkins Hospital*. Through the kindness of Dr. Williams we were enabled to examine cultures from this case, and to confirm his diagnosis of the presence of the bacillus aerogenes capsulatus :

The autopsy was made in cold weather, thirty-one hours after death. The pelves and calyces of both kidneys were much dilated and contained pus. There was also double pyo-ureter. Many small gas cavities were present in the liver (*Schaumleber*) and myocardium. Cover slips from the pus in the renal pelves showed many bacilli like the gas bacillus, and also slender bacilli and streptococci. Gas bacilli and streptococci were found also in cover slips from the liver. The bacillus aerogenes capsulatus was isolated in pure culture from the liver and the pus, and the usual inoculations of rabbits, guinea-pigs, and pigeons were made with the characteristic results which will be described subsequently.

Although the records of the three preceding cases are not so complete as they might be, they furnish proof of the presence in suppurative inflammations of the upper urinary passages and the bladder of the bacillus aerogenes capsulatus in combination with other bacteria. In one of the cases there was gas in the renal pelves and ureters, and it may have been overlooked in the other cases. In one case there was no invasion of the gas bacillus beyond the urinary tract at the autopsy. It is interesting to note the presence of gas cysts in the pelvis of the kidney. The detection of gas bubbles in the left auricle in Case XVII three quarters of an hour after death is highly significant.

The following case is one of gas cysts of the intestine without invasion of the gas bacillus beyond the intestine :

XX. Service of Dr. Osler. Man in hospital for several weeks with choked disk, headache, and other symptoms of cerebral tumour. There was extremely obstinate constipation. Autopsy January 5th, fifteen hours

after death, in very cold weather, the body having been kept in an ice chamber. There was no evidence of post-mortem decomposition. There was a vascular sarcoma the size of a hen's egg in the right frontal lobe of the brain. Congestion of lower lobes of the lungs and bronchopneumonia. Chronic diffuse nephritis. The stomach contained dark-colored fluid. The large intestine throughout contained hard scybalous masses. The intestines were not distended. The peritonæum was smooth, without excess of fluid. There were two areas in the jejunum, ten centimetres apart, each about five centimetres in length, occupied by gas blebs around the whole circumference of the gut. These cysts are visible through the serous coat as a mass of bubbles, and they form projections on the mucous surface. They occupy all of the coats of the intestine. The individual cysts are the size of a pea and smaller. Upon incision they collapse. There is no evidence of inflammation in the intestine. There is no gas in the other organs or in the blood vessels.

Cover-slip specimens show bacilli apparently identical with gas bacilli among the bacteria in the intestinal contents. Within the cysts the gas bacilli are so numerous that other bacteria are not recognised on the cover slips. Cultures from the intestine and from the cysts gave abundant growth of the bacillus aerogenes capsulatus. Cultures from other organs and the blood are sterile.

In the preceding case the invasion of the gas bacillus was limited to the intestinal wall, in which a mass of gas cysts was developed over two circumscribed areas. The bacillus was present in large numbers in the intestinal contents.

The following three cases are examples of the rapid development of gas after death. The first one is of very remarkable character:

XXI. For the report of this case we are indebted to Dr. Berkley, who brought the specimens to the laboratory for our examination. Negro, aged fifty-one, had been an inmate of the epileptic ward of the Bay View Asylum of Baltimore for eleven years. The attacks were of the nature of *grand mal*, and occurred often once or twice daily. On the evening of June 3d he appeared well. At five o'clock the following morning he was found by the attendant unconscious in bed with bubbles of reddish froth issuing from the mouth and nose. He died within twenty minutes of the time that he was found in this condition.

Within an hour after death the body was noticeably swollen and emphysematous. Six hours later the body was still more swollen and emphysematous, and purple blebs of various sizes had appeared over the upper part of the cadaver. The autopsy was held twelve hours after death. No rigor mortis. The body, especially the upper part, is enormously swollen. The eyelids are puffed out and emphysematous. The skin of the scalp is emphysematous. The tissues of the scrotum and penis are greatly distended with gas. The abdomen is enormously distended and tympanitic.

The extremities, shoulders, neck, and chest crackle on pressure, and there are large numbers of blebs. A large amount of gas free from putrefactive odour escapes upon opening the peritoneal cavity. The gas burns in jets of bluish flame with detonation on applying a lighted match. The large veins contain blood mixed with gas. The heart is distended with blood and gas bubbles. The blood vessels generally contain gas, but in relatively smaller amount than the subcutaneous tissues. Gas bubbles are present in the liver and spleen in large numbers. On section of the kidneys, blood mixed with gas bubbles oozes from the cut vessels. The lungs are congested, œdematous, of dark-red colour, and the gas which can be squeezed from the lungs burns with bluish flame. The stomach and intestine are only moderately distended with gas. Cover slips and cultures from all parts showed in very large numbers and in pure culture the gas bacillus which inoculated in pigeons and guinea-pigs caused typical gas phlegmons, not always fatal.

The rapid and diffuse development of gas in the preceding case within a short time after death can not well be explained, in the light of Welch and Nuttall's experiments, otherwise than as referable to a general distribution of the gas bacilli throughout the body during life by the circulation. No lesions were found to indicate the point of entrance of the bacilli into the circulation. We shall refer to this case again in considering the various conditions with which the gas bacillus is associated.

For the notes of the two following cases we are indebted to Dr. W. T. Howard, Jr., Professor of Pathology in the Western Reserve Medical College of Cleveland.

XXII. Man died in the third week of typhoid fever. Autopsy eighteen hours after death. No cadaveric decomposition. There was no emphysema or marked tympanites at the time of death. Emphysema of the subcutaneous tissues, especially of the neck, chest, axillæ, and scrotum, was found at autopsy. The heart and blood vessels generally contained gas. The endocardium and the intima of the vessels were diffusely reddened by imbibed colouring matter. The serous cavities contained gas and blood-stained serum. The mediastinal tissues were emphysematous. The liver and spleen contained gas holes, but were less distended with gas than the kidneys. In the small intestine were typical typhoid ulcers, rarely reaching to the muscle. There was no perforation or peritonitis.

The bacteriological examination by cover slips and cultures gave abundant gas bacilli with the usual reactions in cultures and when inoculated into animals.

XXIII. Man died in the second week of typhoid fever. The day before death there was marked tympanites. Autopsy eight hours after death. No odour or discoloration indicating decomposition. There was no swell-

ing of the body, but on careful examination, slight emphysematous crackling was detected on the outer side of the forearm just below the elbow, at the wrists, and along the outer side of the thighs. No gas in the superficial veins. No gas in the heart or blood vessels, or in any organ except the liver, which was large, pale in colour, and everywhere perforated with small gas holes. The gall bladder and the bile ducts contained a large amount of gas. The walls of the gall bladder and the surrounding connective tissue were emphysematous. There was extensive necrosis of the liver cells found upon microscopical examination. Peritonæum smooth, without excess of fluid. Many typhoid ulcers in the intestine without perforation.

Cover slips showed an enormous number of gas bacilli in the liver. A few were found in the heart. The bacillus aerogenes capsulatus was isolated in culture from the liver, and gave the typical reactions when inoculated into animals.

It is Dr. Howard's opinion—with which we agree—that in the last case the gas bacillus invaded the liver by way of the bile ducts. The large amount of gas in the gall bladder and biliary passages speaks for this view, as we have found that gas is late in appearing in these situations when the gas bacilli are conveyed to the liver by the blood vessels. Although in Case XXII there was far more extensive general development of gas throughout the body, the liver in the present case contained much more gas than in the former. The liver was the only organ which contained gas bubbles, and although gas bacilli had begun to make their appearance in the blood vessels, they had not at the time of the autopsy produced gas bubbles in the blood, although gas had appeared in a few situations in the subcutaneous tissue.

(*To be concluded in the following number.*)

FURTHER RESEARCHES ON THE CLOSURE OF THE CORONARY ARTERIES.

By W. T. PORTER.

(*From the Laboratory of Physiology in the Harvard Medical School.*)

The purpose of this communication is to examine the chief phenomena which follow closure of the coronary arteries, and to inquire into the cause of these phenomena, in the hope of clearing up, if possible, a confusing and not altogether necessary controversy.

I. The Frequency with which Arrest of the Heart follows Closure of the Coronary Arteries.

The closure of the coronary arteries was accomplished in my experiments by stopping the mouth of the artery in the sinus of Valsalva, or by plugging the branches with emboli composed of lycopodium spores, or by ligation of the arteries on the surface of the heart.

The first two methods were employed in twenty dogs. In each case the heart was promptly arrested.

Closure by ligation was done in sixty-seven dogs. The frequency with which the heart was arrested is shown in the following table:

Artery ligated.	Series I.		Series II.		Series III.		Series IV.	
	Arrest.	No arrest.	Arrest.	No arrest.	Arrest.	No arrest.	Arrest.	No arrest.
Ramus circumflexus	4	1	0	2	0	1	3	0
Ramus descendens	4	4	0	7	2	16	5	1
Ramus septi	0	2	0	3				
Coronaria dextra	2	9	0	3				

The reason for the division of the experiments into series will be stated after the consideration of the ligations as a whole.

The summary of the four series of experiments discloses two facts of great interest. The first is that *the frequency of arrest is in proportion to the size of the artery ligated.* The smallest artery of

the four is the arteria septi, the ligation of which has not caused arrest; the next in size is the coronaria dextra, fourteen per cent of its ligations having been followed by arrest; then comes the still larger descendens, with twenty-eight per cent; and finally the circumflexus, the largest artery of all, with sixty-four per cent.

The second fact of interest is that *arrest is least frequent after the ligation of the arteries the preparation of which most injures the cardiac tissues.* In my experience, the preparation of the circumflex is made with less injury to the heart than the preparation of any other artery. It is done without loss of blood, without touching the cardiac muscle, and without any necessary injury of the ventricular nerves, excepting those in the artery itself, presumably of vasomotor nature.* The descendens is less easy to prepare, although this artery, like the circumflex, can usually be laid bare by merely incising the visceral pericardium and connective tissue. The position of the coronaria dextra, lying in a mass of fat which is richly provided with blood vessels and traversed by nerves, makes its isolation much less easy. The ligation of the arteria septi is really a difficult operation. This artery is never seen on the surface of the heart. It is the only artery of the four the preparation of which inevitably lacerates the cardiac muscle. Yet the ligation of this artery never stopped the heart, while the ligation of the circumflex, least difficult of all to prepare without injuring the heart, caused arrest eleven times in eighteen cases. †

Summary of Ligations.

Artery ligated.	No. of cases.	Arrest.	No arrest.	Percentage of arrests.
Ramus circumflexus	11	7	4	64
Ramus descendens	39	11	28	28
Ramus septi	5	0	5	0
Coronaria dextra	14	2	12	14

* Heymanns and Demoor. Étude de l'innervation du cœur des vertébrés à l'aide de la méthode de Golgi. *Arch. biol.*, 1895, xiii, pp. 619–676. W. T. Porter. The Vasomotor Nerves of the Heart. *The Boston Med. and Surg. Journal*, 1896, p. 39.

† The difficulty of reconciling these facts with the idea that the arrest of the heart after ligation of a coronary artery is due to mechanical injury of the heart will be insisted upon in the discussion of the cause of standstill.

Equally interesting facts are revealed by a further analysis of these statistics. It will be noticed that the ligations are presented in four series. The first series was made in Berlin,* the dogs being given curare and, in four cases, morphine; the second was made in St. Louis,† ether alone being used; the third and fourth were done in Boston. In the third, one dog was anæsthetized with morphine and ether throughout the operation, the remainder being etherized until the destruction of the bulb at its junction with the spinal cord made further etherization superfluous. In the fourth series the treatment was as follows:

Date.	Treatment.	Artery ligated.	Result of ligation.
1895.			
Jan. 17	Bulb cut; peptone. ‡	Descendens.	No arrest.
" 31	Morphine; curare; peptone.	"	Arrest.
Feb. 4	" " "	"	"
" 6	" " "	"	"
" 7	" "	"	"
" 12	" " peptone.	"	No arrest.
" 26	" bulb cut; "	Circumflex.	Arrest.
" 28	" " "	"	"
Mar. 1	" " "	"	"

The four series may therefore be divided into two groups. In the first group, comprising Series I and IV, morphine or curare or both were employed; in the second group, comprising Series II and III, neither of these agents was used, with the exception of a single case in Series III, the animals being narcotized with ether or the brain cut off by section of the bulb. The condition of the heart evidently can not have been the same in the two groups, for morphine and curare do not affect the heart as ether or bulbar section do. Compare now the results of ligation in the two groups. It will be seen that the results are not alike.

* On the Results of Ligation of the Coronary Arteries. *The Journal of Physiology*, 1894, xv, pp. 121–138.

† See Ueber die Frage eines Coordinations-centrums im Herzventrikel. *Arch. f. d. ges. Physiol.*, 1894, lv, pp. 366–371.

‡ The peptone was employed to prevent the coagulation of the blood during the measurement of the blood discharged from the left ventricle before and after the ligation of a coronary artery.

Group I, *Comprising Series I and IV.*					Group II, *Comprising Series II and III.*				
Morphine, curare, or both ; sometimes peptone.					Ether or section of bulb.				
Artery.	No. of cases.	Arrest.	No arrest.	Per cent arrested.	Artery.	No. of cases.	Arrest.	No arrest.	Per cent arrested.
Circumflex.....	8	7	1	88	Circumflex.....	3	0	3	0
Descendens....	14	9	5	64	Descendens....	25	2	23	8
Septi.....	2	0	2	0	Septi..... ...	3	0	3	0
Coronaria dextra	11	2	9	14	Coronaria dextra	3	0	3	0

The number of ligations of the circumflex, arteria septi, and right coronary arteries in Group II is so small that I will not use them for comparison with those of Group III further than to point out that they make it probable that the condition of the heart is a factor of importance in the results of ligation. This probability is made a certainty by the comparison of the arrests from ligation of the descendens, the arrests being sixty-four per cent where morphine and curare are used, and only eight per cent where they are not used. The *frequency of arrest is greatly increased by morphine and curare.*

II. The Effect of Closure of the Coronary Arteries on the Blood Pressure and on the Force and Frequency of the Heart Beat.

In my paper On the Results of Ligation of the Coronary Arteries the conclusions were reached that "a gradual and continuous decrease in the height of the intraventricular upstroke appears almost immediately after ligation. When ligation does not cause standstill this lowering of the upstroke is absent or transient. When standstill follows ligation the diastolic intraventricular pressure is invariably increased."

Further experience has supported these statements.

The diastolic rise of pressure is particularly important. The normal mean pressure in the auricles and in the pulmonary veins is known to be very low.* It is probable, therefore, that the pressure in the coronary veins near their mouths is also low. A rise of a few millimetres in the auricular pressure would in that case interrupt

* W. T. Porter. *The Journal of Physiology*, 1892, xiii, pp. 513–553.

the entire coronary circulation, unless overcome by a compensatory rise of pressure in the coronary arteries. This rise in the auricular pressure is certainly present after the ligations which are followed by arrest, for the rise of diastolic pressure which has been shown to take place in the ventricle must be accompanied by a similar rise in the auricle, owing to the free communication between the two chambers during the greater part of diastole. The rise in auricular pressure after ligation is, moreover, not compensated by increased pressure in the coronary arteries. On the contrary, the pressure in the arteries is falling while that in the auricles is rising.

It must be acknowledged, then, that a rising auricular pressure after ligation may at length put a stop to the whole blood supply of the cardiac muscle, and, as this rise is often occasioned by the closure of a single vessel, it is plain that the entire coronary circulation can in fact be interrupted by the ligation of one coronary artery.

This possibly explains the fact that both ventricles, if arrested at all, usually stop at the same instant, no matter which ventricle the ligated artery supplied. But in our present ignorance of the nature of the cardiac co-ordinating mechanism it would be hazardous to insist on this explanation.

The frequency of the ventricular beat is seldom changed when ligation is not followed by standstill. When, however, ligation is followed by standstill, the frequency is usually sooner or later altered. But some hearts beat with unchanged rhythm to the last. In hearts isolated by a modification of Ludwig's method, to be described in the next section, the frequency after ligation is less regular, the explanation of the difference doubtless being that the hearts thus isolated are placed under very unusual conditions.

III. The Effect of Closure of the Coronary Arteries on the Quantity of Blood discharged by the Left Ventricle.

The method employed to measure the outflow from the heart was in the main that used by Pawlow * in Ludwig's laboratory. The

* *Archiv für Physiologie*, 1887, pp. 452–468.

differences were the exposure of the heart, in order to reach the coronary arteries; the closure of the aorta by ligation above the root of the left lung instead of closure by distending a bag introduced into the aorta through the subclavian artery; and the omission of the floats in the measuring cylinders, the change of current which they would have produced by making a contact at the top of the cylinders being secured in my experiments by turning a double key (*Wippe*) when the blood rose to a certain mark on the cylinder. This method of turning the current into first one and then the other of the magnets which control the inflow and outflow from the measuring cylinders was found to be sufficiently exact for the business in hand. The turns of the double key were recorded electrically on the smoked paper. The quantity of blood thrown out by the left ventricle was not essentially changed by ligations that were not followed by arrest. On the other hand, *the quantity of blood thrown out by the left ventricle steadily diminished after the ligations that were followed by arrest.* (Fig. 1.)

Fig. 1.—Showing fall in arterial pressure and diminished output of left ventricle in consequence of the ligation of the circumflex artery. The curve reads from left to right. It is one half the original size. The upper curve is the pressure in the carotid artery. The unbroken line is atmospheric pressure. The next curve is the measurement of the outflow from the left ventricle, each rise and each fall indicating the passage of 50 c. c. of blood into the aorta. The lower line is a time curve in seconds. At * the circumflex artery was ligated.

The details of the experiments are shown in the tabular view.

The Number of Cubic Centimetres of Blood thrown out by the Left Ventricle per Fifty Seconds before and after the Ligation of a Coronary Artery.

Date.	Jan. 17.	Feb. 12.		Jan. 31.	Feb. 6.		Feb. 26.		Feb. 28.		March 1.	
Seconds.	C. cm.	C. cm.	Pulse.	C. cm.	C. cm.	Pulse.	C. cm.	Pulse.	C. cm.	Pulse.	C. cm.	Pulse.
1– 50....	387	482	87	392	405	93	720	130	725	43	315	83
51–100....	387	477	87	333	468	97	675	105	702	47	293	80
101–150....	342	432	..	293	446	98	720	93	743	45	315	81
151–200....	333	392	87	293	423	96	765	96	720	45	315	82
											Ligation of circumflex.	
201–250....	342	378	87	329	455	93	765	94	720	46	297	76
	Ligat. des'n.	Ligation of descendens.		Ligat. des'n.			Ligation of circumflex.		Ligation of circumflex.			
251–300....	351	482	87	446	441	95	648	126	608	37	270	78
301–350....	324	468	82	243	441	96	558	123	486	36	234	75
							Arrest 7 sec. later.					
351–400....	320	441	84	180	423	94	...	...	419	36	221	76
				Arrest.	Ligation of descendens.				Arrest 20 sec. later.#			
401–450....	329	405	..	...	482	99	...	...	...	...	167	67
											Arrest 35 sec. later.‖	
451–500....	315	482†	83	...	405	94						
501–550....	324*			...	387	91						
					Arrest 30 sec. later.‡							

The anæsthetics and peptone employed in the experiments are given on page 48, in the account of the fourth series of ligations.

The results of these experiments are in accord with the measurements of intracardiac pressure already mentioned. The fall of pressure in systole and the rise in diastole are accompanied by a diminished outflow from the left ventricle, while the frequency of beat is usually little changed. These changes in the heart beat, together with the slowing of the systole demonstrated in my first paper, are the fundamental alterations in the activity of the heart which

* The experiment of January 17th continued 2,190 seconds longer. Ligation was not followed by arrest.

† The record of outflow continued until the fourteen hundredth second. No important change could be perceived. The heart beat, in all, 2,900 seconds, and was then stopped by the clotting of the blood in the measuring cylinders.

‡ During these 30 seconds 225 cubic centimetres were thrown out.

During these 20 seconds 135 cubic centimetres were thrown out.

‖ During these 35 seconds 113 cubic centimetres were thrown out.

usher in the loss of co-ordinating power and final arrest of the heart after the ligation of the coronary arteries.

We must now inquire the cause of these phenomena. Two explanations have been offered. The changes in the heart beat are produced either by the sudden arrest of the nutrition of a sufficiently large part of the heart or by the mechanical injury done the heart in the operation of ligation. I shall show that the closure of a coronary artery puts an end to the nutrition of the area which it supplied and that the phenomena which follow the ligation of the coronary arteries are not due to the mechanical injury of the heart.

IV. The Ligation of a Coronary Artery puts an End to the Nutrition of the Area which it supplied.

That the closure of a coronary artery puts an end to the nutrition of the area which it supplied is an accepted truth in pathology, so well established by observation and direct experiment that it would be unnecessary to mention it here had not some recent writers neglected the obvious bearing of this fact on the correctness of their own hypotheses. The present state of pathological opinion regarding this matter is assuredly fairly expressed in such works as Ziegler's *Allgemeine Pathologie*, eighth edition, 1895. I find there the following statements: "Anæmic infarcts occur principally in the spleen, heart, kidneys, and retina" (page 175). "Anæmic infarcts are formed when the vascular area closed possesses no communications through which blood can be received; the area then remains bloodless and dies" (page 173). These words certainly represent the general belief of pathologists regarding the anæmic infarcts of the heart.

Hæmorrhagic infarcts are also sometimes, though very rarely, present in the heart. The infarcted area is then infiltrated with blood. Investigators differ as to the source of this blood, some believing it to be derived from neighbouring capillaries, others through back flow from the veins. But investigators are agreed that in no case is the circulation re-established. On this point there is no dis-

pute. The blood which slowly works in from neighbouring vessels "stagnates" * and can not preserve the life of the part.

The present unanimity of opinion as to the entire interruption of the circulation in the area of infarction rests, it must be remembered, not merely on post-mortem observations, but also on the consequences of the experimental closure of terminal arteries in the living animal. So also is the speedy death of the infarcted area a matter of direct observation. The experimental closure of terminal arteries in the living animal for the purpose of studying the infarcts formed has until very recently been done on organs other than the heart—the kidney, for example. The experimental production of infarcts in the heart has been attempted, so far as I am aware, only by Kolster and myself, in each case with complete success.

Kolster † tied a small branch of the ramus descendens in the dog and kept the animal alive from one day to seventeen months. The microscopical examination of the hearts of dogs killed twenty-four hours after ligation showed a "typical coagulation necrosis in the area of the artery ligated." Kolster remarks that "nothing else could have been expected, for Weigert (Virchow's *Archiv*, Bd. lxxix) took these very cardiac infarcts as a type of coagulation necrosis. All the alterations which Weigert gives as characteristic of the necrosis established by him as the consequence of cutting off the blood supply are to be found here" (page 24). In dogs kept longer alive the progressive alterations were traced from day to day and from month to month.

My own experiments ‡ were made on the main coronary trunks, the descendens, circumflex, or arteria septi, and in some cases more than one of these arteries, being ligated close to their origin, and the animals allowed to live. Two dogs in which the descendens was ligated a few millimetres from its origin lived respectively four days and fourteen days and a half. Characteristic anæmic infarcts were found occupying the anterior part of the septum and that part of the anterior wall of the left ventricle which adjoins the interven-

* Ziegler, *loc. cit.*, p. 145.

† *Skand. Arch. f. Physiol.*, 1893, iv, pp. 1–45.

‡ *Arch. f. d. ges. Physiol.*, 1894, lv, p. 366–371.

tricular furrow. The infarcted areas were examined macroscopically, thin sections being made with a sharp knife.* The area infarcted was completely degenerated, except close to its borders. These experiments show (1) that the arteries ligated are terminal arteries;† (2) that the rapid closure of a coronary artery is followed by the death of the part which it supplies; and (3) that each part of the heart wall receives its blood supply essentially from one main artery; for example, the branches of the circumflex do not contribute any supply of importance to the area embraced by the branches of the descendens. This last fact is in accord with Cohnheim's statement that each coronary artery in the dog's heart keeps to its own boundaries, and does not, as a rule, pass into the field of another artery. Kolster's observation that the infarcted area contained spots of normal heart tissue depends probably on his having tied a small branch and not a main trunk.

The attempts made by Michaelis‡ and others to overthrow the great mass of pathological observations and the unequivocal testimony of infarcts experimentally produced will hardly commend themselves. Michaelis concludes that the coronary arteries are not terminal arteries, because a skilful injector (Wickersheimer) has succeeded in making an injection liquid pass from one artery, through communicating branches, to another. No one, so far as I know, has ever denied that one coronary artery could be injected from another. Cohnheim himself makes special mention of the passage of liquids through the communicating branches under pressure.# Every one agrees that the coronary arteries anastomose. It is not the absence, but the character of the anastomosis that is the basis of the present pathological teaching. The incontestable fact is that the anastomosis is too slight to permit a collateral circulation

* The well-known descriptions of the microscopic appearance of infarcts in the human heart and the very minute description of infarcts in the dog's heart, published by Kolster a few months before my work, seemed to make a microscopical examination superfluous.

† I do not speak here of the immediate object of the experiments published in the *Archiv f. d. ges. Physiologie*—namely, the demonstration of the absence in the infarcted area of any nerve centre essential to the co-ordination of the contraction of the heart.

‡ *Zeitschrift für klinische Medicin*, 1894, xxiv, pp. 270–294.

Virchow's *Archiv*, 1881, lxxxv, p. 509.

sufficient to keep a vascular area alive after the closure of the artery which supplies it.

The argument of those who rely on injections to prove the absence of terminal arteries appears to premise that one artery can be injected from another only when arterial communications exist, and that terminal arteries do not have arterial communications. The first premise is incorrect. Thin injections can be forced through any blood vessels. The second premise is inexact. Terminal arteries may possess arterial anastomoses, provided the resistance in the communicating vessels is high. The idea of terminal arteries is physiological, not anatomical. Terminal arteries differ from other arteries in that the peripheral resistance in the anastomosing vessels is too high to be overcome by the normal blood pressure in any of the arteries of which the communicating vessels are branches. Hence the rapid closure of any terminal artery cuts off the nutrition of its own capillary area because sufficient blood for the life of the area can not be sent through the communicating vessels on account of the high resistance in them. The resistance in the communicating vessels, and not their size, is the factor of first importance. The circumstance on which Michaelis lays so much stress—namely, that one coronary artery can be injected from another—has long been known, and has no force against the pathological and experimental evidence of the terminal nature of coronary arteries.

I conclude, then, that the rapid closure of a coronary artery puts an end to the nutrition of the area which it supplied.

If now it can be proved that the changes in the heart beat produced by the ligation of a coronary artery are not the result of the mechanical injuries of the operation, it will follow that these changes are the consequence of a sudden interruption of the nutrition of the heart.

I shall determine, first, the consequences of extensive mechanical injuries of the tissues surrounding the coronary arteries; second, the effects of the mechanical injury done the heart in preparing a coronary artery for ligation; and last, the results which follow the closure of the coronary arteries without mechanical injury.

V. The Consequences of Extensive Mechanical Injuries of the Tissues surrounding the Coronary Arteries.

The experiments* about to be described were made on dogs poisoned with curare. I have already shown that curare increases the frequency of arrest after ligation (page 49). Notwithstanding the unfavourable influence of curare, arrest took place but once in ten cases of sudden and violent crushing of the periarterial tissues. The method of operating will be seen in the following example:

Experiment July 7, 1892.—Dog; 8,100 grammes; curare; artificial respiration. The descendens was quickly and carefully freed from its bed at a point thirteen millimetres from the aortic origin of the left coronary artery. Only the visceral pericardium and the connective tissue over the artery were injured. A strongly curved, threaded needle was now passed into the cardiac muscle, entering on one side of the interventricular furrow opposite the place where the artery lay bare and emerging on the opposite side. The distance between the point of entry and of exit was about twelve millimetres. The needle, thanks to its curve, passed through the cardiac muscle three millimetres beneath the artery. The thread was now brought back underneath the artery. Thus the periarterial tissues were included in the ligature, but the artery was not included. This ligature was drawn tight, crushing the tissues embraced in it and dragging, doubtless, on many ventricular nerves. The intraventricular pulse, recorded by a Gad-Cowl manometer, was counted during four hundred and sixty-five seconds. Before the ligation, the frequency per ten seconds was twenty-three; after the ligation, the frequency varied from twenty-one to twenty per ten seconds. There was no arrest.

The number of experiments after this method was ten. In five the tissues about the descendens alone were crushed, in three those about the circumflex alone, and in two those about both arteries. In nine of the ten animals the heart continued to beat; slight irregularity is noted in three, and in one an increase both in force and frequency. The following case of double crushing is especially noteworthy:

Experiment July 27, 1892.—Prepared periarterial tissues around the circumflexus about six millimetres from the aortic origin of the left coronary artery and about the descendens on a line with the anterior edge of the pulmonary artery. Drew both ligatures tight. No great irregularity.

* These experiments were done in the University of Berlin in 1892.

In the case in which arrest followed the crushing, the periarterial tissues about both circumflex and descendens were prepared. As in all experiments, the tissues were included for at least ten millimetres, the thread at its deepest point being about three millimetres below the surface. On drawing the circumflex periarterial ligature tight, the heart fell into fibrillary contractions.

The result of this series of deliberate, extensive injuries to the cardiac muscle and nerves lying about the coronary arteries is most instructive. It shows clearly that too much has been made of the supposed effects of such injuries on the heart, and that they by no means explain the consequences of ligation of the coronary arteries. This conclusion will become more and more probable as we proceed.

VI. Mechanical Injury of the Heart in preparing the Artery for Ligation.

The mechanical injuries said to be the cause of the phenomena which often follow the closure of a coronary artery are produced by the preparation of the artery for ligation. They can hardly be produced by the injury to the artery itself in the act of ligation, for not even the most ardent advocate of mechanical injury would, I presume, venture to claim that the crushing of the arterial wall alone seriously affects the heart beat. The extent of the injuries produced by the preparation of the artery have surely been greatly exaggerated. The coronary arteries, excepting the arteria septi, can be prepared without the injury of a single muscle fibre, or even in many cases the loss of a single drop of blood. The superficial nerves which are sometimes pulled upon or torn during the operation are, so far as is known, centripetal nerves, the section and direct stimulation of which does not cause the effects the explanation of which we seek.*

The statistics that are available do not show that the preparation of the artery has any serious consequence whatever. It will be remembered that Fenoglio and Drogoul† clamped or ligated a coro-

* Wooldridge, L, *Archiv für Physiol.*, 1883, pp. 522–541. McWilliams, *Journal of Physiology*, 1887, viii, p. 298.

† *Archives italiennes de biologie*, 1888, ix, p. 49.

nary artery in fifty dogs without once seeing the phenomena that precede arrest. The notes of nearly one hundred ligations made by me show that the only effect of the preparation of an artery is the occasional dropping of a beat. The changes in intracardiac pressure and the engorgement of the heart which form the unvarying introduction to the arrest that often follows ligation have never been observed. Several operations were made during the continuous recording of the blood pressure in the carotid artery by a Hürthle manometer. The number of dropped beats and the state of the blood pressure were thus accurately determined. One of the curves

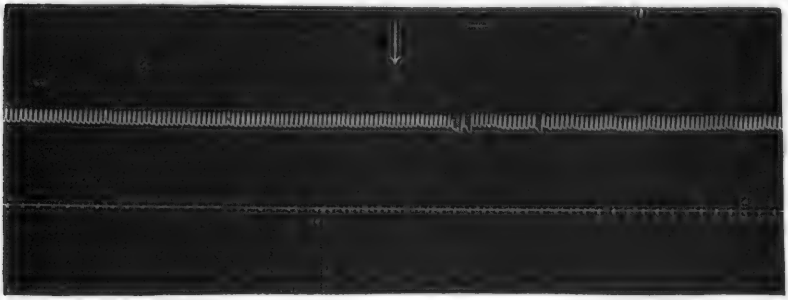

Fig. 2.—Showing the character of the irregularity sometimes occurring during the preparation of a coronary artery for ligation. The curve reads from left to right. Upper line is carotid pressure and pulse curve. Lower line is atmospheric pressure and time in seconds. At ↓ the preparation of the descendens for ligation was begun.

is here reproduced. (Fig. 2.) The records thus secured confirmed the observations with the unaided eye. An example of these experiments will be given.

Experiment September 20, 1895.—Dog; ether; tracheotomy; division of bulb near spinal cord; artificial respiration; continuous curve of carotid pulse and pressure. Prepared descendens for ligation thirteen millimetres from the aortic origin of the left coronary artery. Occasionally a dropped beat during the preparation; nothing more. After seventy-three seconds the ligature was drawn tight. Speedy arrest of the heart with tumultuous fibrillary contractions and strong auricular pumping.

In addition to the facts just mentioned, the reader will remember those demonstrated in the first section of this paper—namely, that the frequency of arrest is in proportion to the size of the artery

ligated, and is least frequent after the ligation of arteries the preparation of which most injures the cardiac tissues. These observations can not be reconciled with the mechanical-injury explanation.

I am therefore able to conclude that the necessary mechanical injury of the heart in the preparation of an artery for ligation may cause a slight irregularity in the ventricular stroke, but that this irregularity is almost invariably transient and unimportant.

VII. The Closure of the Coronary Arteries without Mechanical Injury.*

It has now been shown that extensive sudden injuries of the tissues lying about the coronary arteries rarely arrest the heart, or do more than cause a passing irregularity. It has been shown also that in nearly one hundred consecutive ligations of the coronary arteries not one case was found in which the preparation of the artery for ligation materially affected the beat of the heart. The methods thus far employed can not, however, wholly exclude the influence of mechanical injury, because ligation can not be accomplished without such injury. It is possible, nevertheless, to close a coronary artery without the slightest injury either of the artery itself or of the surrounding tissues. This is done by passing a glass rod through the aorta into the aortic opening of the coronary artery, or by plugging the branches of the coronary arteries by means of emboli composed of lycopodium spores. The first method is carried out as follows:

The dog is etherized and tracheotomized, the bulb destroyed near its junction with the spinal cord, and artificial respiration begun. The left first rib is exposed near the sternum, and a small opening made into the chest in the angle formed by the rib and the sterno-cleido-mastoid muscle. Through this opening, the left internal mammary artery is easily secured. The first five ribs on

* The first mention of my method for this purpose was made in the *Centralblatt f. Physiol.*, 1895, No. 16, p. 481. See also the reply to R. Tigerstedt in a later number of the same year.

the right side and the first three on the left side are now exposed by a straight incision along the margins of the sternum. Stout ligatures are passed with a large bent aneurism hook around the eight exposed ribs some distance from the sternum, and around the left fourth and fifth and the right first, second, and third ribs near the sternum. Another strong ligature is tied around the sternum between the third and fourth pairs of ribs. The ligated ribs and the upper part of the sternum may now be removed. It is best to begin with the fifth rib on the left side, cutting along the outer line of ligatures, raising the ribs as soon as possible, and securing the right internal mammary artery by reaching underneath the sternum. The pericardium should be slit a little to the right of the left phrenic nerve, and sewed to the margins of the chest opening with three or four stitches. The innominate artery is found beneath the inner border of the innominate vein, ligated as near the neck as possible, a rubber band placed under it near the cardiac side of the ligature, a compression forceps applied about fifteen millimetres below the ligature, and the previously oiled glass rod introduced into the artery through as small a hole as possible. The artery should now be compressed about the rod by tying the rubber band, which should press tightly enough to prevent bleeding along the rod, but not so tightly as to hinder the operator in moving the rod. The rod is passed down toward the left anterior sinus of Valsalva. As soon as the resistance of the aortic valve is felt, the rod is withdrawn a little and its head made to traverse the aortic wall near the bottom of the sinus. The opening of the left coronary artery will presently be found. Into this the rod is passed and fastened in place by tying a ligature around the innominate artery, including the rod. A finger of the left hand placed very gently over the left coronary artery will readily determine whether the rod is in the right place. The rod is 210 millimetres long, the head and neck being of the size and shape represented in the accompanying figure. (Fig. 3.) The position of

Fig. 3.—Lower portion of glass rod (actual size) used to plug the left coronary artery.

the rod was always carefully examined post mortem by the method to be described in the account of the following experiment of September 20, 1895.

Plugging the left coronary artery during life is not a very difficult operation. I have attempted it in nineteen dogs, each time with complete success. The closure of the artery was always promptly followed by arrest. Fibrillary contractions were present in every case but one.

It seems hardly possible that the gentle introduction of the glass rod into the artery could in any way injure the artery, and, in view of the facts presented in former sections of this paper, it is wholly improbable that any slight injury which might be thus inflicted would stop the heart. Nevertheless, even this remote possibility can be excluded.

Testimony against injury by the rod is furnished by the experiment of September 20:

Experiment September 20, 1895.—Dog; 9,500 grammes; ether; tracheotomy; artificial respiration; section of bulb at its junction with the spinal cord.

The heart being exposed, the glass rod was passed through the innominate artery into the supposed trunk of the left coronary artery. The head of the rod was distinctly felt by a finger placed over the circumflex artery. The handle of the rod was now tied in place. The expected standstill did not follow, nor could any irregularity in the heart beat be detected. After waiting some time for any sign of heart failure, and perceiving none, the descendens was laid bare. The descendens pulsated. It was now ligated thirteen millimetres from the aorta. Speedy standstill followed, with tumultuous fibrillary contractions and strong auricular pumping. The heart was greatly distended.

Autopsy: Find head of rod in the circumflex artery, nine millimetres from the aorta. Open circumflex just beyond the head of the rod. No blood escapes. Raise the pressure in the aorta. Still no blood escapes; closure perfect. Open descendens on cardiac side of ligature. Blood immediately runs out in a stream. Outflow hastened by raising the pressure in the aorta. Descendens not closed by rod. Find that each artery has a separate origin, the circumflex opening being situated between the middle and left end of the sinus of Valsalva, the descendens near the right end. The circumflex was tightly plugged, the descendens not at all.

In this experiment, then, the plugging of the circumflex artery caused no disturbance of the heart beat. The operation of plugging

was precisely the same in this case as in others in which closure was followed by arrest with fibrillary contractions. The constant arrest after complete closure and the nearly constant fibrillary contractions can not therefore be explained by the mechanical injury done by the rod.

The next experiment is even more instructive.

Experiment September 23, 1895. — Dog; 11,000 grammes; ether; tracheotomy; section of bulb near spinal cord; artificial respiration. The search for the opening of the left coronary artery lasted forty-four seconds. Not a heart beat fell out. Immediately after closure of the artery, the crural blood pressure began to sink, the individual heart beats grew weaker and weaker, and the heart began to swell. At the sixty-fifth second after closure, the blood pressure had fallen from 60 millimetres, Hg., to 25 millimetres. The rod was now drawn back into the aorta. The pressure continued to fall a few millimetres during twenty-two seconds, then rose gradually, reached its former height in fifty-five seconds, and then rose farther to 85 millimetres, after which a slight fall occurred. As the blood pressure rose, the individual beats increased in strength and the heart returned to its normal size. (Fig. 4.)

The artery was now closed again. The result was quite as before. The blood pressure sank, the heart swelled, and the beats were small. After forty-six seconds, the rod was once more withdrawn. All the threatening symptoms disappeared. In fifty-six seconds the heart was working as well as before.

A third and a fourth time was this repeated. The fourth time the artery remained closed fifty seconds. The heart swelled enormously. Its beats were scarcely perceptible in the curve. During 127 seconds, it went from bad to worse. Finally, it remained seven seconds without a beat. Strong massage was then employed, the heart being compressed six times in seven sec-

Fig. 4.—Illustrating experiment of September 23d. The curve reads from left to right. It is one half the original size. The upper line is the pressure in the crural artery. The lower line marks atmospheric pressure and time in seconds. At * the left coronary artery was plugged. At † the plug was withdrawn.

onds. The beat returned. The massage was twice repeated, each time with good results. Finally, the heart beat again strongly and regularly.

Once more the artery was closed and the entire series of these interesting phenomena called back. The artery was shut for fifty seconds. This time, however, the heart beat did not return after the withdrawal of the plug. Massage helped some, but the highly swollen heart could be made to contract but a few times.

If the introduction of the rod had caused the arrest in this experiment through mechanical injury, the arrest would have been permanent. The injury done by the introduction of the rod would not have been undone by its withdrawal.

The observations now to be described appear to be conclusive:

Experiment October 14, 1895.—Dog; 11,500 grammes; ether; tracheotomy; section of bulb near spinal cord. A glass tube, slightly conical at the end, with a diameter of 2·75 millimetres at the outlet, was connected with a bottle of warmed defibrinated ox blood, diluted one half with normal saline solution (0·6 per cent). The bottle was 150 centimetres above the heart. The blood was warmed nearly to the body temperature, and kept thoroughly oxygenated by a constant stream of oxygen. The glass tube was now introduced into the aortic opening of the left coronary artery. As usual, it entered the circumflex, closing the mouth of the descendens. The blood was at once turned on and allowed to flow for eight minutes. During this time 318 cubic centimetres flowed through the circumflex. The heart continued to beat. There were no symptoms of arrest. At the end of eight minutes the blood was shut off. Arrest followed in 120 seconds. The left coronary artery was found tightly plugged by the glass tube, which extended into the circumflex, stopping the descendens with its side.

Experiment October 17, 1895.—Dog; 9,500 grammes; ether; tracheotomy; section of bulb. At 11.39 A. M. the descendens was ligated about one centimetre from the aorta. No arrest. At 12.15 P. M. the glass tube used in the preceding experiment was passed through the aorta into the circumflex artery, and warm defibrinated, oxygenated, diluted ox blood was sent through the tube at a pressure of 140 centimetres (blood). The heart was at first stimulated, the blood pressure rising. The blood pressure returned then to its former level or a little less. Every alternate beat was weak. After four minutes, 114 cubic centimetres of blood mixture having run through, the oxygenated blood was suddenly replaced with carbon-monoxide blood. The heart became within four seconds very irregular. After twenty-eight seconds the carbon-monoxide blood was turned off and oxygenated blood allowed to flow again, but it was too late. Four seconds thereafter arrest with fibrillary contractions occurred.

The blood was prepared by dividing 960 cubic centimetres of defibri-

nated ox blood into two parts and diluting each with an equal volume of normal saline solution (0·6 per cent). Through one part carbon monoxide was passed during forty-five minutes; through the other, oxygen. Both mixtures were afterward warmed in stoppered flasks to near the body temperature. The carbon monoxide was made from oxalic and sulphuric acids, and the carbon dioxide removed by passing the gas through two flasks filled with a strong solution of sodium hydrate. The gas was then conducted through a wash flask, and finally into the blood. Passing the purified carbon dioxide through barium hydrate gave no precipitate, showing that the carbon dioxide had been wholly removed.

The experiments just cited show that the presence of the tube in the coronary artery is not the cause of the arrest with fibrillary contractions observed after closure of the artery. Were this arrest due to a mechanical injury by the tube, it could not have been prevented by circulating oxygenated blood through the tube.

If the glass tube did not cause arrest though mechanical injury, the arrest that followed the introduction of the glass rod could not have been due to mechanical injury, for the tube was fully as large as the rod.

Arrest with fibrillary contractions follows the stopping of the coronary arteries with lycopodium spores. The method is illustrated by the following experiment:

Experiment December 17, 1895.—A dog was etherized, the bulb severed, and artificial respiration begun. The left carotid and left subclavian arteries were tied near their origin. Ligatures were placed around the common origin of the right subclavian and carotid arteries and about the aorta below the left subclavian artery, but were not drawn tight. The cannula of a syringe filled with the warmed, defibrinated, oxygenated blood from the same dog was tied into the left carotid artery. This blood contained a great quantity of lycopodium spores. The hitherto open ligatures around the right subclavian and carotid arteries and the aorta were now drawn tight, and at the same moment the lycopodium blood was driven through the carotid into the aorta. There was now but one outlet for the blood in the arch of the aorta—namely, the outlet through the coronary arteries. These vessels were at once distended with the blood mixed with lycopodium. At this instant the ligatures were loosed again, forty-two seconds after they had been drawn tight. The circulation was free to go on as before. About twenty seconds later the heart, although beating strongly, suddenly became irregular, stopped short, and fell almost at once into violent fibrillary contractions. At the autopsy, the smaller coronary branches ramifying over the surface of both ventricles were seen to be plugged with masses of lycopodium.

In this experiment the heart was not once touched by the operator. The heart itself closed the coronary arteries. It is well known that a brief ligation of the aorta is borne by the dog's heart without apparent injury. In this dog the heart still contracted strongly after the aortic ligature was loosed. There can therefore be no thought of mechanical injury in this experiment.

The inquiries regarding the relation between mechanical injury of the heart and the results that follow the rapid closure of a coronary artery have now been answered. It has been shown (1) that sudden extensive injuries of the tissues lying about the coronary arteries rarely arrest the heart or do more than cause some irregularity; (2) that in almost one hundred consecutive ligations of the coronary arteries not one case was found in which the preparation of the artery for ligation materially affected the beat of the heart; and (3) that closure of a coronary artery without mechanical injury produces all the phenomena that have been ascribed to the mechanical injury of the heart.

Hence, of the two conceivable reasons for the changes in the action of the heart after closure of the coronary arteries, one—the mechanical injury of the heart—is excluded. The phenomena brought on by closure of the coronary arteries are due, therefore, to the sudden stopping of the nutrition of a considerable part of the heart.

VIII. The Changes in the Heart Beat that follow the Opening of the Great Arteries.

The principal factor in the creation of the prevailing difference of opinion regarding the cause of the phenomena that follow closure of the arteries has been a serious misconception—namely, that the phenomena in question differ essentially from the changes produced by the sudden stopping of the supply of blood to the heart muscle, when the great arteries are opened freely. When the carotid arteries or other large branches of the aorta are opened, the co-ordinated contractions of the heart grow weaker and weaker until they finally disappear. Very different from this is the picture that Cohnheim and others have drawn of the changes following the closure of the

coronary arteries. In the widely circulated paper of Cohnheim and von Schulthess-Rechberg,* it is stated that the ligation of a principal branch of the left coronary artery has no immediate influence on the action of the heart, neither the frequency nor the character of the pulse nor the blood pressure being altered during the first thirty to forty seconds, and often still longer after ligation. Only as arhythmia becomes pronounced does the blood pressure sink, and then but slightly. And this is the more surprising, for the strongly beating although irregular heart now suddenly stops and the arterial pressure falls swiftly to the abscissa (page 513).

I have already shown the incorrectness of Cohnheim's generalizations. The force of the heart beat, the height of the blood pressure, the power of the ventricle to discharge its contents, and the amount of blood thrown into the aorta, are all progressively diminished by those ligations that are followed by arrest, and this chain of alterations is seen to begin within a few seconds after the closure of a sufficiently large branch. They do not begin instantly because the capillaries are not instantly emptied, the more so for the reason that the pressure from behind is largely cut off by the tying of the artery. The heart, then, is not struck down by a single blow in the midst of full activity. On the contrary, the fatal result is always preceded by the changes mentioned above. The irritability of some hearts, however, is so great that they are brought to a stop before the characteristic alterations have gone very far. The smallness of the alteration observed by Cohnheim is probably to be explained by the high irritability and consequent speedy arrest produced by the curare that he used, a drug which increases the sensitiveness of the heart to closure of the coronary arteries, as I have pointed out above.

The rise of diastolic pressure so constantly present after the closure of the coronary vessels is not seen when the anæmia of the heart muscle is brought about by bleeding from the great arteries. In the former case the high peripheral resistance against which the ventricle must pump prevents the enfeebled organ from emptying

* Virchow's *Archiv*, 1881, lxxxv, pp. 502–536.

its contents into the aorta; in the latter the peripheral resistance is almost nothing, and even weak contractions expel the contents of the ventricle with ease. The ventricle, moreover, receives less than the usual quantity of blood through the auricle. The difference in the height of diastolic pressure can not therefore be used in support of the idea that the results of anæmia due to closure of the coronary arteries are essentially different from those due to anæmia from great hæmorrhage; and the alleged essential difference in the other symptoms that precede arrest does not exist, as I have already clearly demonstrated.

A second argument in favour of a difference between anæmia from hæmorrhage and anæmia from closure of the coronary arteries is sought in the statement of Cohnheim and von Schulthess-Rechberg that "no sort of stimulus will make the dog's heart give even a single contraction after standstill has occurred" (page 516). The impossibility of restoring the heart beat after standstill has once occurred, the authors insist, shows most conclusively that the arrest can not be due to anæmia. According to them, the ligature about the coronary artery can be loosed as soon as standstill has occurred and blood forced through the coronary arteries by massage of the ventricles, but the heart does not beat again (pages 521–525). This argument has no longer any force, for it has been shown by Arnaud,* Hédon and Gilis,† Langendorff,‡ and myself# that the heart brought to a standstill by bleeding from the great vessels can be made to beat again for long periods by forcing blood through the coronary arteries, and I have proved in the preceding section of this paper that a heart arrested by plugging the coronary arteries can recover its power of co-ordinated contraction when the plug is withdrawn and the heart is massaged. There is then no essential difference here.

It is charged, especially by Langendorff,‖ that fibrillary contrac-

* *Arch. de physiol.*, 1891, pp. 396–400.
† *Comptes Rendus Soc. de biol.*, 1892, pp. 760, 761.
‡ *Arch. f. d. ges. Physiol.*, 1895, lxi, pp. 291–332.
# *The Boston Med. and Surg. Journal*, 1896, p. 39.
‖ *Loc. cit.*

tions are absent when anæmia of the heart muscle is produced by opening the great vessels.

Fibrillary contractions are simply inco-ordinated, irregular contractions of individual cardiac muscle cells or groups of cells. When a dog is bled from the carotid arteries, the contractions of the heart, as has been already said, grow feebler and feebler, until they finally disappear. Immediately or very soon thereafter three kinds of contractions can be seen in the heart—first, contractions in various parts of the ventricles, very often not co-ordinated with each other, and of small area; second, small, sharply marked, narrowly circumscribed twitchings here and there on the surface of the heart. These two kinds of movements are apparently the last traces of the action of a co-ordinating mechanism that is no longer master of the whole heart. Third, slight fibrillary contractions, commonly very faintly, sometimes almost or altogether unrecognizable, at other times quite unmistakable.

There is, I believe, no fundamental difference between these various contractions and the pronounced fibrillary contractions heretofore considered in this paper. They are identical in that they are all circumscribed contractions of a part of the heart wall appearing after a temporary or permanent loss of the power of general co-ordination. They are further alike in that they can be made to give place to co-ordinated contractions. The restoration of the co-ordinated beat is easy in all mammals yet investigated, except the dog. Tigerstedt, Michaelis, and others declare that fibrillary contractions of the dog's heart are invariably fatal. In taking this position, they assume a fundamental difference between the heart of the dog and the hearts of nearly related mammals. They oppose, moreover, their negative results to the positive observations of McWilliam,* who more than once has seen fibrillary contractions in the dog's heart give place to regular co-ordinated beats; and they do this in the face of the fact that no one, so far as I am aware, has attempted to restore the dog's heart, after fibrillary contractions have begun, by the systematic employment of an artificial circulation through

* *Loc. cit.*, p. 299.

the coronary arteries. It is evident that without this, recoveries will be rare indeed.

It can not be admitted, then, that fibrillary contractions differ in kind from the irregular, inco-ordinated contractions that appear after standstill brought on by opening the great vessels.

The difference in degree is certainly great. The cause of this difference lies probably in the fact that after closure of the coronary arteries the heart continues to work against a high peripheral resistance. The co-ordinating mechanism of the strongly working anæmic heart soon gives way, while the less highly specialized contractility of the muscle fibres is still considerable. Pronounced fibrillary contractions are then possible. The heart made anæmic by opening the arteries, on the contrary, contracts against almost no peripheral resistance. It does little or no work. The co-ordinating mechanism is only gradually borne down. The contractility of the cardiac muscle cell sinks with it, and when arrest has finally come the contractility remaining is not sufficient for outspoken fibrillary contractions.

An example of this is seen in the single case in which my closure of the left coronary artery with the glass rod produced arrest without the ordinary tumultuous fibrillary contractions. The heart of this dog was brought slowly to standstill, and the contractility, or, perhaps one should say, the irritability, of the muscle fibres was probably so much reduced thereby that decided fibrillary contractions were no longer possible.

The changes in the heart beat that follow the opening of the great arteries are therefore, in my opinion, not fundamentally different from those produced by the sudden closure of the coronary arteries.

THE EFFECT OF ODOURS, IRRITANT VAPOURS, AND MENTAL WORK UPON THE BLOOD FLOW.*

PLATES I–VII.

BY T. E. SHIELDS, Ph. D.

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INTRODUCTION AND DESCRIPTION OF APPARATUS.

ATTEMPTS have been made from time to time to determine the effects produced by various sensations on the circulation of the blood. In 1877 a series of experiments was conducted by MM. Couty and Charpentier, under the direction of M. Vulpian,† in the hope of obtaining a more exact determination of these disturbances. They used curarized dogs with artificial respiration and recorded the results on a kymograph in the usual manner by means of a mercury manometer in connection with an artery. On stimulating the various organs of special sense they obtained cardio-vascular reactions similar in many respects to those usually obtained by stimulating sensory nerves, but differing markedly from these in their greater variability in both form and intensity under the same stimuli.

Both pneumogastric nerves were then severed. Sensory stimulation continued to produce its usual effect on arterial tension, but ceased to produce any effect on the heart. This convinced them of the fact that the heart disturbances and vasomotor changes were mediated by distinct mechanisms.

The cerebral cortex of fresh subjects was then shut out by pressure, by injection, and by light doses of chloral hydrate. In every case stimulation of the organs of sense failed to produce any effect, either on the heart or on arterial pressure, while the direct stimula-

* A thesis submitted to the Johns Hopkins University for the degree of Ph. D.

† De l'influence des excitations des organs des sens sur le cœur et sur les vaisseaux (recherches exp. faites dans le lab. de M. Vulpian), note de MM. Couty et Charpentier, presentée par M. Vulpian. *Comptes Rendus*, t. lxxxv, No. 3, p. 161.

tion of a peripheral nerve, such as the sciatic, continued to produce its usual effect. From these facts they concluded that the cardio-vascular disturbances accompanying sensory stimulation are not due directly to sensation itself, but to a contingent cerebral activity which they called emotion.

Experiments of this nature, however valuable in themselves, will hardly be regarded as sufficient data from which to draw conclusions as to the nature and extent of the cardio-vascular changes accompanying sensations in man. Even if it be granted that cerebral activity is the immediate cause of the cardio-vascular changes in both man and dog, it does not follow that these changes will be the same in the two cases.

In 1880 Dogiel * attempted, by means of the plethysmograph, to study the effects of music on the circulation of the blood in man. The apparatus used contained so many sources of error that even the meagre results obtained can scarcely be regarded as reliable. It is to Mosso's † researches that we are chiefly indebted for what definite information we possess concerning the correlation of cerebral function and cardio-vascular changes in man, but our literature on the whole subject of the changes produced in the circulation in response to various sensations is still very meagre. This is particularly true of olfactory sensations. The present research was undertaken in the hope of throwing some light on this problem.

The form of plethysmograph devised by Mosso was first used, and the volume changes in the arm recorded on a drum kymograph; but it soon became evident that several sources of error would have to be eliminated before accurate measurements could be obtained. The possibility of moving the arm into or out of the cylinder during the experiment must be removed. It is not sufficient to request the subject to sit quietly, nor even to support his head and arm, as Dogiel ‡ did. The voluntary effort required to sit immovable is itself a disturbing factor in the circulation of the blood. Besides, it is impos-

* *Archiv für Anat. und Physiol., Physiol. Abthlg.*, 1880, S. 420 ff.

† Kreislauf des Blutes im menschlichen Gehirn. Von A. Mosso, Leipzig, 1881.

‡ *Op. cit.*, pp. 419 et 429.

sible to prevent movement of the arm for any considerable time, since normal respiration moves the arm. Mosso's * plan of suspending the cylinder from the ceiling and allowing it to follow the movement of the arm is much better, but even here the inertia of the cylinder filled with water offers sufficient resistance to the movements of the arm to produce quite a perceptible error. I think it probable that the respiratory waves † often obtained by the plethysmograph are due in greater measure to this movement of the arm than to the changes in its volume of blood. During the early part of the present research I frequently obtained marked respiratory waves, but in every case I was able to trace them to this movement of the arm, and when the movement was prevented the respiratory waves were very much diminished if not entirely suppressed.

A second source of error lies in the fact that every increase of pressure in the cylinder tends to force the arm out of it, and every diminution of pressure exerts an opposite influence. The play of these opposing forces is felt very distinctly on the inclosed arm during each pulse beat, and when the resulting movement of the arm is prevented the record of the pulse wave is very much amplified. This source of error is still more marked in sudden large vasomotor changes, nor can it be eliminated by making the rubber sleeve so tight that it will not slip on the arm; for apart from the two obvious objections to this mode of procedure—that the arm should be oiled ‡ to prevent it from absorbing water, and that a tight sleeve interferes with normal circulation—the elastic sleeve itself lengthens and shortens in response to changing pressure in the cylinder.

There is still another source of error connected with this mode of closing the cylinder which remains even after all movements of the arm into or out of the cylinder have been prevented. If the arm does not fit the mouth of the cylinder snugly the portion of the elastic sleeve between the arm and the cylinder will yield to each change of pressure in the cylinder. The panting of this membrane in response to the pulse may be seen by the unaided eye. If the mouth

* *Op. cit.*, p. 44. ‡ The oil, of course, increases very much the tendency to slip.
† Dogiel, *op. cit.*, p. 130.

of the cylinder be large enough to allow the elbow to enter, it will usually be found much too large to fit the upper arm. This was very conspicuously the case in the cylinder used by Dogiel.* If the cylinder terminates over the large muscles of the forearm, as it does in Mosso's arrangement, it can be made to fit the arm snugly, but another source of error appears. Every movement of the inclosed fingers withdraws muscle from the cylinder or introduces muscle into it.

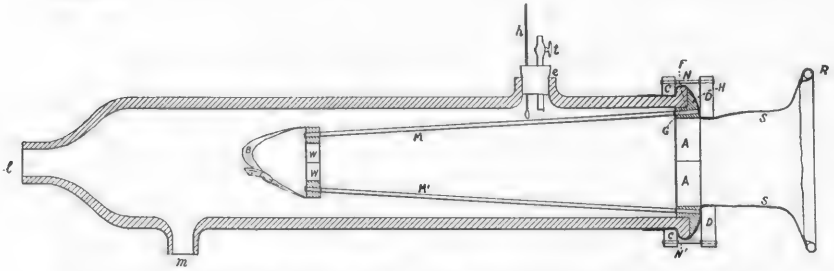


FIG. 1.—S, rubber sleeve; R, ring cemented into end of rubber sleeve; A, arm ring; G, shoulder in same; H, bevelled face of arm ring supporting rubber sleeve; C, D, rings making arm holder rigid to cylinder; N N′, rods connecting the rings C and D; W, wristlet; B, band for same; M, *e*, *l*, smaller openings in arm cylinder; *h*, thermometer; *t*, stopcock; M M′, metal rods, making wristlet rigid to arm ring.

Fig. 1 is a sketch of a device for removing these sources of error The same is shown in Plate II. The lettering is the same in both.

One end of a suitable-sized rubber sleeve, S, is drawn over the arm cylinder and fastened in position by a ligature. A metal ring, R, large enough to pass readily over the flange is cemented into the other end of the sleeve. By the aid of this ring the sleeve is easily doubled back over the flange of the cylinder, where it remains until the arm has been put into position in the cylinder. A hard-rubber wristlet, provided with a hinge and clasp, fits round the wrist. A soft band, B, passes from one side of this wristlet between the thumb and index finger and is buckled to the other side of the wristlet as tightly as comfort will permit. A second hard-rubber ring, A, provided with hinge and clasp, fits the arm above the elbow. This ring is rigidly connected with the wristlet by two metal rods, M, M′. A

* *Loc. cit.*

shoulder, G, is cut into one face of this ring so as to make it fit into and against the mouth of the cylinder. The face, H, of the ring is shaped so as to keep the rubber sleeve taut down to the point where it meets the arm. When the arm has been introduced into the cylinder and the arm ring fitted into its place, the rubber sleeve is drawn down over it. Two hard-rubber rings, C and D, rigidly connected by two metal rods, N, N′, and provided with hinges and clasps, are then fastened outside the rubber sleeve. One of these rings, C, rests against the flange of the cylinder; the other, D, fits tightly against the outer surface of the arm ring.

This arm holder reduces to a minimum, if it does not entirely remove, the sources of error discussed above. All movements of the elastic sleeve are rendered impossible. The arm holder is made absolutely rigid to the cylinder. The wristlet, with its tight band between thumb and index finger, prevents the arm from moving in the holder. As the inclosed arm can not bend, no muscle can be withdrawn from the cylinder or introduced into it.

The arm was now found to change in volume about one cubic centimetre at each pulse beat. This introduced a new difficulty in the recording apparatus. It will be remembered that in Mosso's * plethysmograph the volume changes were recorded by means of a test tube suspended in a beaker of water or some liquid of suitable specific gravity. The test tube was counterpoised by a weight carrying the recording needle and connected with the test tube by two threads passing over a pulley. However well this arrangement may serve for recording slow changes, its inadequacy for recording such changes as we are here dealing with is evident. The introduction and withdrawal from the test tube of a cubic centimetre of water fifty to one hundred times a minute sets up considerable agitation in the water in the beaker, where every motion is a source of error. Besides, the system has a period of its own which sometimes neutralises the pulse effects and at other times combines with them to produce greatly exaggerated excursions of the test tube, as happens in

* MM. A. Mosso et P. Pellacani. Sur les fonctions de la vessie. *Archives italiennes de biologie*, vol. i, p. 98.

the production of tone beats. The great inertia of the system also prevents it from recording accurately such rapid changes in volume.

The method adopted by Bowditch * of suspending the test tube from a spiral spring is more convenient. This removes the error which, in Mosso's plan, arises from the motion of the water in the beaker. It also materially lessens the inertia of the system. But the great inertia still remaining, and especially the periodicity of the spring, compelled me to seek some other method of recording. I had recourse to a water manometer of large bore connected with the cylinder by a wide, indistensible tube. A light and buoyant float † was used to carry the recording needle. This system, being rigid, and having no period of its own except that due to the float, gave a fairly accurate record of the changes occurring in the volume of the arm; but it introduced a new source of error which compelled me to abandon its use. The level of the water in the manometer tube determines the pressure which the water in the cylinder exerts on the arm. At the beginning of the experiment the manometer may be set so as to exert the proper pressure. But in the course of the experiment every change in the volume of the arm will cause a change of pressure on its surface. The arm sometimes changes twenty or thirty cubic centimetres in volume in a few seconds. This, of course, causes a corresponding change in the level of the water in the manometer tube, which in turn alters very considerably the pressure on the arm; nor can this factor be neglected. I found that the change of pressure due to an alteration of a few centimetres in the level of the water in the manometer caused a very large change in the volume of blood in the arm. That is what we would expect from the low blood pressure in the capillaries and veins.

Lehmann ‡ gives the following account of the apparatus used by him in investigating the effects of sensory stimulation. I quote

* *Proceedings of the American Academy*, May 14, 1879.

† Howell and Warfield. *Studies from Biological Laboratory, Johns Hopkins University*, vol. ii, p. 235.

‡ Die Hauptgesetze des menschlichen Gefühlsleben. Von Dr. Alfr. Lehmann, Leipzig, 1892.

his description in full, as I shall have occasion to refer to the results obtained later on.

"Wir haben nun die Aufgabe auf die Bestimmung der Atembewegungen, des Herzschlages und des Volumens eines einzelnen Gliedmasses, z. B. eines Armes, reduciert. Die erste Bestimmung ist mittels des Pneumographen leicht auszuführen, während die beiden letzten sich mittels Mosso's Plethysmographen zusammen ausführen lassen. Dieser besteht aus einem am einen Ende geschlossenen Rohr, das eben weit genug ist, den Arm zu umschliessen, der durch einen Gummiärmel wasserdicht mit dem Rohr verbunden wird. Durch ein Seitenrohr wird das ganze Rohr, nachdem der Arm in die rechte Stellung gebracht ist, mit Wasser gefüllt, und hierauf wird das Seitenrohr durch einen Gummischlauch mit einem Mareyschen Schreibapparat (*tambour enregisteur*) in Verbindung gebracht. Jede Veränderung des Armvolumens bewirkt eine Hebung oder Senkung des Wassers, durch welche die Luft im Schlauch und im Schreibapparat beeinflusst wird, und die Bewegung wird dann vom Stift des Schreibapparats auf eine rotierende Walze (das Kymographion) gezeichnet."

Lehmann's apparatus seems to be a Mosso hydrosphygmograph * in which the bottle for keeping the pressure constant has been omitted so as to compel the tambour to register both pulse and vasomotor effects. In both these forms of apparatus the pressure on the arm is too high. The air cushion is at the top of the cylinder. The whole arm is consequently under a positive pressure of some centimetres of water. The under surface of the arm can scarcely be under a pressure of less than twelve centimetres. If the cylinder be large enough to extend above the elbow, as it should do in order to escape a source of error mentioned above, this pressure is likely to be still higher.

The form used by Lehmann introduces another grave source of error. In dispensing with the pressure bottle, the tambour and cylinder are rendered a closed system in which every change of volume in the arm alters the pressure on its surface. If, as some-

* Diagnostik des Pulses. Von Dr. A. Mosso, Leipzig, 1879.

times happens, twenty-five or thirty cubic centimetres of blood be added to the volume of the arm, the distention of the membrane of the tambour necessary to make room for this increased volume of blood would exert a considerable pressure on the arm.

Besides the sources of error enumerated in connection with each of the forms of apparatus discussed above, there is a defect common to them all. We have seen that the changes in volume of blood in the arm are due to at least two distinct sources—changes in the heart's action and changes in the calibre of peripheral vessels. It is therefore highly desirable that the record of these two effects be kept as distinct as possible. Whenever, as is the case with each of these forms of apparatus, the same writing point is made to record the pulse and the gross volume changes, these effects partially mask each other and do not stand out as distinctly as they should. This is illustrated very well by many of the curves published in Lehmann's work. I have a large number of similar curves in my own possession which I obtained by the use of these forms of apparatus.

From what has been said, it is evident that an efficient apparatus for recording the volume changes in the arm must at least meet the following three requirements: First, the pulse and vasomotor effects should be recorded separately; second, the apparatus for recording the rapid and rhythmic changes of volume due to the pulse should have very little inertia and no periodicity; third, the pressure exerted on the arm should be as nearly normal as possible, and must remain constant during all changes of volume which may occur in the arm. These requirements are met fairly well by the following arrangement:

A long metal tube, A′ (Plate I), is slipped over an upright, U, to which it is fastened by a clamp, C. The top of the upright is held in position by two metal rods, M, M′, which make it rigid to the wooden screen interposed between the subject and the kymograph. A short metal tube, B, is supported in a vertical position by two clamps, D, D′, which make it rigid to the upper end of tube A. One end of a long close spiral, S, of No. 5 piano wire is slipped over the lower end of the tube, B, and fastened to it by a clamp, E. Two

wires, F, F′, pass down from the free end of the spiral, and are fastened by two small binding screws to a light hard-rubber ring, R, one inch in diameter. By suitable cork collars, test tubes of various sizes may be suspended from this ring. The slipping of the wire under clamp E permits the length of spiral to be so adjusted that the level of the water in the suspended test tube will remain constant while the quantity of water in the test tube varies. One arm of a long glass siphon, G, is passed down through tube B, thence through the centre of the spiral S, and dipped a few millimetres beneath the surface of the water in the test tube T. The siphon is centred and held in position by six set screws passing through tube B. An indistensible tube, H I, connects the other end of the siphon with the arm cylinder. The four-way J interposed in this tube will be described later.

This is essentially the same as the apparatus described by Bowditch, to which reference has already been made. Its inertia unfits it for recording the changes in volume due to the pulse. Its periodicity combining with the rhythmic pulse at times neutralizes the pulse effects, and at other times combines with them to produce greatly exaggerated excursions of the test tube. The pulse and vasomotor effects also partially mask each other. The pulse effects are the disturbing factor in each case; if they be removed from the test tube and recorded separately, the instrument is well adapted for registering the vasomotor effects.

By the following device this separation of the pulse record from the vasomotor record may be attained, the pulse wave being recorded by a tambour the tension of which remains practically constant except for the variation with each pulse wave and the vasomotor changes being recorded by the test tube and spiral spring already described, which are so arranged that only the slow vasomotor changes in the volume of the arm cause any perceptible movement of the system, the quicker changes due to the heart beat disappearing from this record. A wide glass piston tube (K, Fig. 2 and Plate I; P, Plate II) is supported in a vertical position beside the arm cylinder, with the bottom of which it communicates freely. The

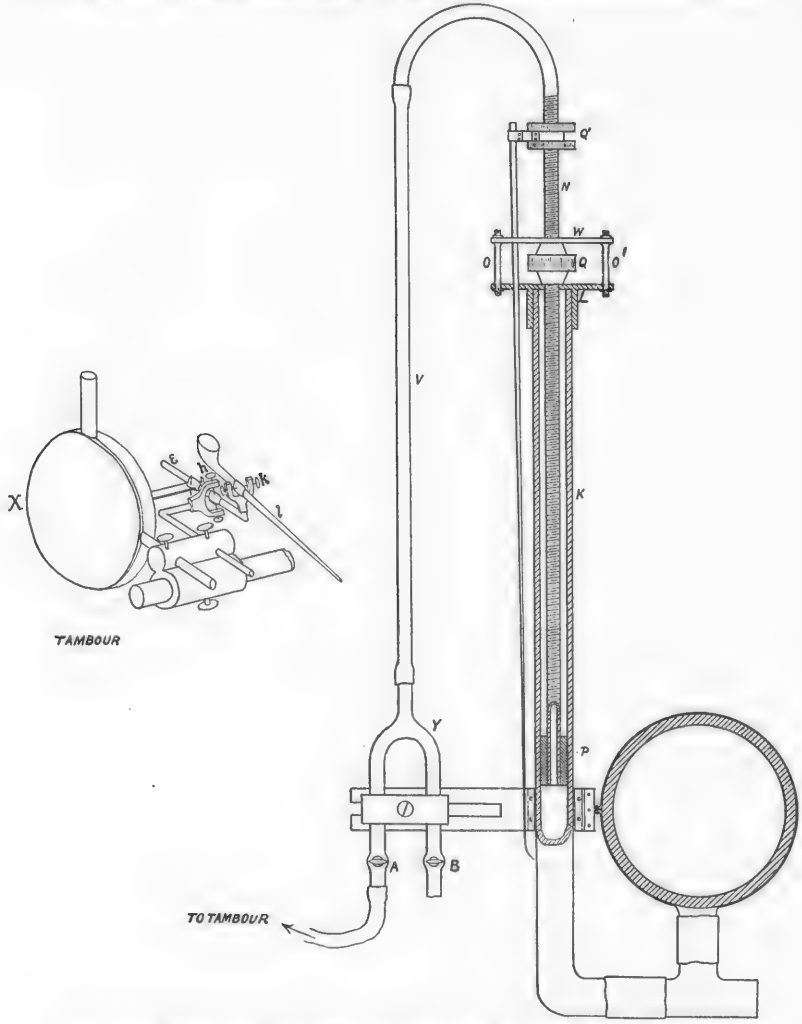


FIG. 2.—K, piston tube;* P, piston; N, piston rod; Q, milled nut for adjusting level of piston; Q′, milled nut for adjusting indicator; *g*, metal cap of piston tube; W, washer, giving support to nut Q in lowering piston; O, O′, rods supporting washer; *v*, tube connecting with tambour; Y, T-way; A, B, stopcocks on same; X, tambour; *e*, tambour lever carrying horizontal pivot; *k*, horizontal pivot; *h*, vertical pivot.

* The vertical piston tube allows the pressure of the water on the arm to be regulated at pleasure. The piston in this tube serves to keep the volume of the air between the water and the tambour constant.

upper end of the tube is incased in a metal cap, L (Fig. 2), which is perforated to allow free play to the hollow piston rod, N, and free communication between the air in the upper end of the piston tube and the atmosphere. From this cap two metal rods, O, O′, parallel with the piston tube extend upward about one inch and hold in place a washer, W, which encircles the piston rod. Between the washer and the cap a milled nut, Q, fits on the piston rod, which is threaded from end to end. The piston rod, N, is a metal tube one eighth of an inch in diameter, which extends through an otherwise air-tight piston, P, in the piston tube. A small indistensible tube, V, connects the upper end of the piston rod with a Marey tambour, X. A T-way, Y, provided with two stopcocks, A, B, interposed in the course of this tube, permits connection between the air cushion below the piston and the tambour or the atmosphere to be made or broken as desired. A milled nut, Q′, fitting the piston rod above the washer, carries an indicator, which passes down through a perforation in the washer, thence through a perforation in the flange of the metal cap, and terminates below the level of the piston. The distance between the point of the indicator and the lower surface of the piston measures the vertical height of the air cushion in the piston tube. It can be set at any desired distance by means of the nut Q′. Since the indicator is carried by the piston rod, the distance between its point and the piston remains constant whatever alterations may be made in the level of the piston. The level of the water in the piston tube when the air cushion is in communication with the atmosphere indicates the pressure exerted by the water on the surface of the inclosed arm. For convenience in registering this pressure a centimetre scale is affixed to the side of the piston tube.

The arm cylinder is of the usual form (see Plate II). A flexible metal band, Y, encircles the cylinder near one end and is closed over the upper surface of the cylinder by a nut and bolt. To this bolt are also fastened two chains which pass round the cylinder in opposite directions, and thence through a ring, *d*, which is suspended some distance above the cylinder. The chains are doubled back and hooked on themselves at desired lengths. The other end of the

cylinder is suspended in a similar manner from the same ring. These chains serve to keep the cylinder in a horizontal position and to prevent it from rotating. A fifth chain, from which the ring *d* is suspended, passes through a pulley fastened to the ceiling, thence down to a ratchet which is supported at convenient height. By turning this ratchet the cylinder is raised or lowered to suit the comfort of the subject.

Besides the opening through which the arm is introduced, the cylinder is provided with three other smaller orifices. Through one of these, *l*, situated on the end of the cylinder, communication is made with the suspended test tube used to record the vasomotor changes. A second orifice, *e*, provided with a tight-fitting rubber stopper through which two perforations have been made, is located on the upper surface of the cylinder. A glass stopcock, *t*, is fitted into one of these perforations. The bulb of a thermometer, *h*, is introduced through the other. On the under surface of the cylinder is a third orifice, M, into which is fitted one branch of a large T-way. A second branch of this T-way communicates with the lower end of the piston tube. The third branch is connected with the tube *k* for filling and emptying the cylinder.

I have found the following method convenient for filling and emptying the cylinder, for regulating the temperature and pressure of the water, and for adjusting the instrument:

The piston is raised or lowered by turning the milled nut Q until the point of the indicator stands at the level to which it is desired to bring the water in the piston tube. Two supply jars, P, Q (Plate I) —one filled with hot water, the other with cold water—are placed on a shelf attached to the screen above the level of the test-tube siphon, G. Two siphons connect these jars with two branches of a large three-way stopcock, *w*, which permits the hot and cold water to be mixed in the desired proportions. The bulb of a thermometer for indicating the temperature of the water is placed in the tube, which conducts the water from the three-way *w* to one branch of a second large three-way, *z*. A tube connects a second branch of this three-way with the arm cylinder; another tube leads from its third

branch to the sink. This three-way allows the water to pass from the three-way *w* to the cylinder, or from the cylinder to the sink, or from a hydrant tap to the supply jars, as may be desired.

The four-way J, each branch of which is provided with a stopcock, is made rigid to the tube A, at a level somewhat lower than that of the arm cylinder. One of these stopcocks (1) opens into the test-tube siphon ; a second (2) communicates with the arm cylinder; a third (3) is connected by a siphon with one of the supply jars; a tube from the fourth (4) leads to the sink.

When the subject's arm has been coated with vaseline and placed in the arm cylinder, as directed above, stopcock *t*, on the upper surface of the arm cylinder, is opened to allow the air to escape. Stopcocks A and B (Plate I and also Fig. 2) are closed to cut off connection between the air cushion of the piston tube and the atmosphere and tambour. Stopcocks 1 and 2, on the four-way, are opened, and stopcocks 3 and 4 closed. Three-way stopcocks *w* and *z* are adjusted so as to allow water of the proper temperature to enter the arm cylinder. When the cylinder is within a few cubic centimetres of being full the supply of water from this source is shut off, and a strong clamp placed on the supply tube at the point where it meets the cylinder, to prevent any possible error that might otherwise arise from leakage or from distention of the supply tube. Stopcock 3, on the four-way, is now opened and water allowed to flow into the cylinder until it is filled, when the stopcock at the top of the cylinder (*t*, Plate II) is closed to prevent air from re-entering the arm cylinder. Stopcock 2, on the four-way, is closed at the same moment to prevent increase of pressure on the arm while the test-tube siphon is being filled. When the test tube is partially filled, stopcock 3 is closed and stopcock 2 opened. If the test tube is too full at any time, water may be drawn off by opening stopcock 4. Stopcock B is now opened, thus establishing free communication between the air cushion and the atmosphere. The water in the piston tube will now rise to the same level as that in the test tube. Tube A′, carrying the test tube and its appurtenances, is slowly raised or lowered on the upright until the level of the water in the piston tube reaches

the point of the indicator. Stopcock B is then closed and stopcock A opened, thus putting the air cushion in communication with the tambour. The lever of the tambour will now give a magnified record of the changes in the level of the water in the piston tube. If the resistance offered by the long narrow tube, H I, to the rapid passage of water between the test tube and the arm cylinder is not sufficient to prevent the changes of volume due to the pulse from moving the test tube, this resistance is increased to the desired extent by partially closing stopcocks 1 and 2 on the four-way. The small and rapid changes of volume due to the pulse are consequently recorded by the tambour alone, while the slower and larger volume changes, freed from pulse waves, are recorded by the motions of the test tube, on the same principle that the height of the tide is recorded by the level of the water in the vertical pipe of the tide gauge, while the separate waves are prevented from influencing this level by the resistance which the holes in the bottom of the pipe offer to the rapid passage of water.

This form of apparatus reduces very much the sources of error discussed above. The suspended test tube is entirely freed from the error due to the periodicity of the spring. The inertia of this system ceases to be a grave source of error owing to the slowness of the motions which it is now required to execute. The tambour, relieved for the most part from the disturbing factor of large volume changes, gives a fairly accurate record of the pulse waves. The chief source of error remaining is the pressure exerted by the water on the arm. It is, of course, impossible to have an arm immersed in water under perfectly normal pressure. I have found the conditions to be most favourable when the level of the water in the piston tube is that of the middle of the arm, the lower half of the arm being then under a positive pressure of a few centimetres of water, and the upper half under a like amount of negative pressure.

Each pulse wave is accompanied by a slight change of pressure on the arm, due to the resistance which the water in the cylinder offers to the entrance of the wave. This resistance is made up of several factors: First, the inertia of the volume of water to be

moved; second, the force of gravity to be overcome in slightly raising the level of the water in the piston tube; and third, the resistance offered by the air cushion in the piston tube to change in the level of the water in the tube. The last-named resistance is due to the tension of the membrane of the tambour and to the inertia and friction of the recording lever. These sources of error are quite small, but they still exist, and should be taken into account in calculating results. The resistance offered by the air cushion has been rendered very slight by reduction in the weight and friction of the recording lever, and by the delicacy and low tension of the tambour membrane. The inertia of the mass of water to be moved may be reduced somewhat by so changing the form of the arm cylinder as to diminish the distance between the surface of the water in the piston tube and the point where the expansion of the inclosed arm first occurs. The wider the piston tube, the less the change in the level of the water caused by the pulse wave; but the pulse recorder becomes proportionally more sensitive to vasomotor changes. When there is a sudden large change in the volume of the arm, the vasomotor recorder can not take care of it rapidly enough. There is, in consequence, a temporary variation in the level of the water in the piston tube, which interferes somewhat with the pulse recorder and changes the pressure on the arm. The influence of this disturbing factor, which I have not yet been able entirely to eliminate, will receive attention when we come to discuss the results.

In the early part of the investigation, I used a Ludwig drum kymograph, with smoked paper for recording the curves. It soon became evident, however, that this plan of recording presented another grave difficulty.

Spontaneous changes, or at least changes not due to sensory stimulation, were continually appearing in both vasomotor and pulse curves. It was, therefore, necessary to have a continuous record of the changes going on in the arm for some time before and after stimulation, in order to be able to differentiate them from any sensory reactions that might appear. This is impossible with the Ludwig drum kymograph, for if it be made to travel fast enough to

bring out the pulse curve, the paper will be used up in three or four minutes, and it will be necessary to stop and put on fresh paper. An ink record on a continuous band of paper is evidently what is needed. The chief difficulty in obtaining such a record is the resistance to be overcome in moving the vasomotor and pulse pens. I was unable to reduce this sufficiently in the case of vertical kymographs for continuous paper, and accordingly built a horizontal kymograph, the general arrangement of which is shown in Plate I. By special contrivances for suspending and moving the pens the resistance was reduced within the limits of that offered by the smoked paper.

A light plate of aluminium foil is cut in the form of a cross. The two ends of the transverse beam widen out and are bent so as to clasp tightly the tube of a glass pen. The ends of the other beam of the cross are bent into hooks, from one of which a thread passes over a pulley adjusted to the side of the kymograph, and is held taut by a small weight. From the hook on the other end of the beam a thread passes under a pulley adjusted to the other side of the kymograph, and thence vertically to a violin key, which turns in a cork holder cemented to the bottom of the test tube used for recording the vasomotor changes. This key is turned until the pen rests over the centre of the paper. A thread fastened to the bulb of the pen passes vertically to a similar violin key, which is supported in proper position. The tube of the pen, at a point somewhat beyond where it leaves the holder, is bent downward through an angle of about 45°. The key to which the vertical thread supporting the bulb of the pen is fastened is turned until the point of the pen just touches the paper. The pulleys are so adjusted that the upper portion of the pen will be about horizontal. The thread passing between the pulleys is horizontal also, except for the sag in the middle, caused by the weight of the pen. As the pen moves from either pulley toward the other it describes a curve, due to the sag in the horizontal thread. This curvilinear motion is neutralized in the point of the pen by the pendular motion of the bulb, due to the thread from which it is suspended, the factors in this adjustment

being the extent of the sag, the length of the pendular thread, and the respective distances of the point and bulb of the pen from the horizontal thread. The extent of the sag is controlled by the size of the weight on the end of the thread. The length of the pendular thread is regulated by raising or lowering the key holder from which it is suspended. The distance of the ends of the pen from the horizontal thread is controlled by moving the pen in its holder.

The friction between the point of this pen and the paper is very small, since the point need not actually touch the paper, the capillary attraction between the paper and the meniscus being sufficient to cause the ink to flow if the pen be properly made. But even when the point of the pen actually touches the paper, it does so very lightly. If the point of the pen be lifted by a finger until the sag leaves the horizontal thread, half of the weight of the tube of the pen will rest on the finger. If the finger be now gradually lowered, the weight on it will be found progressively to diminish and finally to disappear, having been thus shifted to the horizontal thread. This gives very great buoyancy to the point of the pen and reduces friction to a minimum.

The pulleys are large and very light, and are pivoted in a manner similar to the balance wheel of a watch. Their friction is thus rendered very small. The inertia due to the pen, its aluminium holder, and the light weight on the end of the horizontal thread, is relatively a very small addition to that of the test tube partially filled with water; but the inertia of this whole system, large as it is, has very little influence on the accuracy of the record, owing to the slowness of the motions which it describes.

The tambour, x (Fig. 2 and Plate I), of the pulse recorder is supported with its membrane vertical. The motions of this membrane are communicated to one end of a light aluminium lever, e, which just after passing through a pivot, h, whose axis is vertical, bends upward at a right angle, and after running in this direction about one inch supports a second pivot, k, whose axis is horizontal and perpendicular to the long axis of the paper. A light glass pen, l, with a long tube is passed through this pivot and fastened to it when

the bulb of the pen is directly over the axis of the first pivot. The motion of the membrane will now impart a magnified horizontal movement to the point of the pen, while its bulb, being in the axis of the pivot, only undergoes a very slight rotary motion. The second pivot allows the pen to move freely in a vertical plane. Ink is now poured into the bulb of the pen until the point of the pen barely rests on the paper. The friction here is very slight.

The pens for the pneumograph (to record the respiratory movements), the time marker, and the signal marking the time of application of the stimulus, are pivoted in a slightly different manner, but one which involves the same principle. The bulb of the pen with the ink which it contains acts in each case as a counterpoise to the long tube of the pen, thus causing the point to rest on the paper as lightly as desired. A horizontal movement is imparted to the point of the pen by a fork of the tambour lever or of an armature of an electro-magnet, as the case may be. For this purpose the pen must be free to move round two axes, one vertical and the other horizontal.

The points of the five pens are adjusted so as to fall in a line perpendicular to the long axis of the paper. The test tube used in the vasomotor recorder was of such a calibre that three cubic centimetres of water moved it one centimetre. The paper travelled from right to left; the time record is nearest the test tube, and consequently at the bottom of the paper. Movement of vasomotor or pulse pen toward the bottom of the paper represents diminishing volume of arm, and conversely. I used the time record as base line in calculating the volume changes. If d represents the initial distance in centimetres between the point of the vasomotor pen and the base line, and d' its distance in any subsequent position, then $(d' - d) \times 3 = V$, where V is the volume change in cubic centimetres. If at any time during the experiment the test tube is becoming overfilled, stopcock 2, Plate I, on the four-way, is closed and stopcock 4 opened until the desired amount is drained off. Stopcock 4 is then closed and stopcock 2 opened, and the fact noted on the paper. The point of the pen, of course, records the volume drawn out, which must be added in cal-

culating subsequent changes. If the test tube is in danger of being emptied, water is introduced through stopcock 3 in a similar manner, and the amount subtracted in all subsequent calculations. Specimens of the curves traced by this instrument may be seen in Plate III.

Being thus enabled to obtain what I believed to be a fairly accurate record of the changes which actually occur in the volume of the arm, I proceeded to study the influences which determine these changes in the hope of being able to eliminate some of them and to differentiate the changes which would remain from any odour reactions I might obtain.

I first determined empirically what temperature of water and what pressure on the arm would maintain the most constant volume of blood in the arm. The most favourable pressure, as has already been stated, is that which is exerted when the level of the water in the piston tube was about that of the middle of the arm. When I used pressure lower or higher than this the arm exhibited a tendency to increase or diminish in volume. The proper temperature of water I found to be between 30° and 34° C, according to the temperature of the room and of the arm of the subject. I found that this temperature kept the volume of the arm more constant than any other, and I found also that the temperature of the water in the cylinder remained constant throughout the experiment, whereas, if the temperature be higher at the beginning, it will sink during the experiment, and conversely, if the initial temperature be lower.

After the experiment had been continued for about half an hour, large and frequent changes of volume usually occurred, which I attributed to the discomfort of the subject, who up to this time had been seated on a wooden chair. These disturbing factors were removed by putting the subject in an invalid chair which could be adjusted to any desired position. He could rest here comfortably, with all his muscles as completely relaxed as if he were in bed. This rendered the volume of the arm much more constant. But the most conspicuous of the disturbing factors still remained. This is the mental reaction. Apparently, every thought that flits across the

subject's mind records its presence by a change of volume in the arm. Talking in the room, the entrance of a stranger, the striking of a clock, or a noise in an adjoining room, is usually accompanied by a change of volume in the arm, and this sometimes even when the subject is asleep. This is what was to be expected from the correlation which Mosso has shown to exist between cerebral function and the volume of the arm.* Nor do I see any way in which this disturbing factor can be eliminated while working with sensory stimulation. The only course open seems to be to confine it within as narrow limits as circumstances will permit, and to endeavour by control experiments to differentiate what mental reactions may remain from the sensory reactions under investigation.

The odours were at first administered to the subject by holding a bottle under his nose and allowing him to smell its contents. This was the plan pursued by Lehmann,† but it is open to several obvious objections. The disturbance of mental equilibrium occasioned by such a procedure is itself sufficient to condemn it. Any sudden or unexpected movement of the operator produces a mental reaction in the subject. On the other hand, I soon found that when he anticipated my movement a reaction appeared. When he saw me reaching for the odour bottle, even when I did not apply the stimulus, a reaction appeared which was often very similar to that accompanying the sensation itself. It is evident, then, that the subject should be isolated, and everything that could attract his attention as far as possible removed. To attain this end a screen was placed between the subject and the kymograph, so as to prevent him from perceiving the recording apparatus or any of my movements, and a special apparatus was devised for administering the odour without exciting or disturbing the subject.

The apparatus used for applying the odours is shown in Plate I. By means of an aspirator, *a*, attached to a hydrant tap, air and cold water are mixed together and poured into a large bottle, *t*. The water is drawn off by a siphon. The air is forced out through a

* Kreislauf des Blutes im mensch. Gehirn. † *Op. cit.*, p. 82.

small tube, *b*, which connects with a coil of lead pipe immersed in a warm bath of constant temperature. This gives us a constant current of washed air of any desired size at a constant temperature and in constant hygrometric condition. A tube, *w*, conducts this current of air into a metal pipe, *y*, fastened to the front of a drawer in the kymograph table. A series of thirteen metal stopcocks open along the upper surface of this pipe. In the front of the drawer is arranged a series of thirteen bottles, each containing a small portion of odoriferous substance. A pair of small glass tubes perforate the stopper of each bottle and terminate at its lower surface. One of each of these pairs of tubes connects with a corresponding stopcock on the metal pipe. Each of the other tubes communicates with a corresponding glass tube which passes down through the bottom of the drawer, bends over to the screen, and runs up to about the level of the subject's head, where it terminates after passing through the screen. Thirteen flexible tubes connect these glass tubes with thirteen short metal tubes which are screwed into a terminal plate, the odour plate, Z′, and end on a level with its distal surface. A tube, 14, which does not make connection with any odour bottle, leads directly from the stopcock on the end of the metal pipe to a fourteenth metal tube in the odour plate. This permits a current of odourless air to be administered when desired.

The odour plate (Z′, Plate I and Fig. 3) is a copper disk, eight inches in diameter, supported from its centre on a universal holder, which allows it to be adjusted in any desired position. Through this plate are bored five series of fourteen holes each, arranged in five concentric circles. The odour tubes terminate in the inner circle of holes. The lumen of a short portion of the terminal end of these metal tubes is less than half the diameter of that of their proximal end. Thirteen of these tubes are provided with electric valves constructed so as to work noiselessly and to leave the face of the plate as free as possible. This prevents the subject from perceiving the opening or closing of the valves, except by his sense of smell, and removes from the face of the plate all objects to which odours might cling.

Fig. 3 shows the construction of one of these valves. A metal disk, A, faced with rubber dam is placed in the large end of the tube. The diameter of the disk is less than the large lumen of the tube and greater than its small lumen. A small wire, *b*, passes from the centre of this disk out through the small lumen of the tube, and a few millimetres from the face of the plate is fastened to a metal rod, *c*, which, after running outward parallel with the face of the plate for a short distance, bends back at a right angle, passes through a perforation in the plate, and is adjusted by two nuts to one end of an armature lever, *d*. This armature lever runs outward parallel with the plate, and terminates over an electro-magnet, *e*. It is supported on a pivot, *b'*, situated a short distance centrally to the magnet. A small spiral spring, *g*, fastened to the odour plate is adjusted

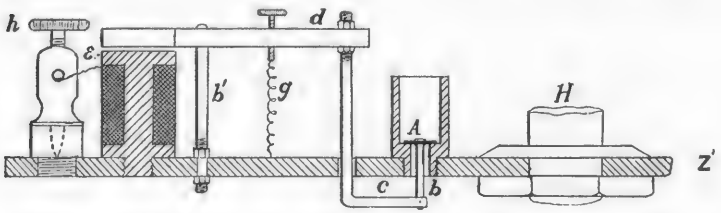


Fig. 3.—Z, terminal plate (odour plate); H, holder; A, metal disk for closing odour tube; *b*, wire from centre of disk A to rod C; C, rod connecting wire of valve to armature; *d*, armature lever; *e*, electro-magnet; *b'*, pivot of armature lever; *g*, spiral spring from armature lever to terminal plate; *h*, insulated binding screw.

to the central arm of the lever by a small nut and bolt, which allow the tension of the spring to be regulated at pleasure. One wire of the electro-magnet makes contact with the plate; the other passes to an insulated binding screw, *h*, fastened to the plate beside the coil. The tension of the spring is so adjusted as to hold the valve closed except while a current is passing through the magnet, and to prevent the armature from touching the magnet at any time. This prevents noise, and the rubber-dam face renders the valve itself noiseless. The movement of the wire on the face of the plate in opening or closing the valve is so small that it escapes the notice of the subject.

A wire from one pole of a battery makes contact with the metal

pipe on the face of the kymograph drawer. Each of the thirteen stopcocks on the upper surface of this pipe is provided with a platinum contact, which, when the stopcock is open, dips into a corresponding mercury well situated on the upper surface of the drawer between the stopcock and its odour bottle. A wire passes from each of these mercury wells to a corresponding binding screw, thence through the coil of the electro-magnet to the terminal plate. A wire in contact with the copper terminal plate passes to the magnet of the signal pen, and thence back to the other pole of the battery.

The opening of any of these thirteen stopcocks, therefore, sends a constant current of air through the corresponding odour bottle, and, by completing the electric current, opens the terminal end of the corresponding odour tube near the subject's nose, and marks the instant of stimulation on the paper of the kymograph.

Opening the fourteenth stopcock at the end of the metal pipe sends a current of odourless air to one of the odour tubes of the odour plate.

To secure quiet and exclude light stimulation, the subject was at first placed in a separate and darkened room. I soon found that movements of any part of the body might produce a change in the volume of the inclosed arm. It was necessary, therefore, to bring him into a position where I could observe any movement he might make. A screen was placed so as to prevent him from seeing me or the recording apparatus. The room was of course kept quiet, and visitors excluded except where their presence is noted. All the records discussed here were taken by the improved form of apparatus described above.

Discussion of Results

To illustrate the character of the records actually obtained, several specimens (reduced in size) of the tracings upon the kymograph are given in Plate III, Figs. 1, 2, 3. Fig. 1 illustrates the vasomotor reaction obtained in a certain instance by the odour of extract of heliotrope, and Fig. 2 shows the effect of formic acid when inhaled into the nose. The lowermost line gives the time record in

seconds; above this is the pulse record taken by the tambour; and above this the volume curve of the arm upon which the pulse movements are not perceptible. A fall of this curve indicates a shrinkage of the arm due to vaso-constriction. At the top of the tracing the respiratory movements are recorded; and above, the signal pen marks the time of application of the sensory stimulus—i. e., in these cases heliotrope and formic acid. Fig. 3 is a portion of a tracing in which the effect of isobutyric acid was studied. This tracing may be taken for special description, as it illustrates one of the main difficulties met with in interpreting the curves. The subject's arm was in the cylinder about fifteen minutes when this portion of the record was begun. Curve E gives the time in seconds. Ordinates are drawn at every fifth second. The base line F is drawn parallel to the curve E. The pulse curve, D, shows very little change in the heart rate. There are nineteen heart beats in the first fifteen seconds, and about eighteen in each succeeding fifteen seconds. The respiratory curve, B, shows no very great irregularities. The vaso-motor curve, C, shows an increase of 1·2 cubic centimetres in the volume of the arm during the first twelve seconds. Curve A indicates that the odour of isobutyric acid was turned on at the tenth second, but it is evident from the respiratory curve (phase of expiration) that it could not have reached the olfactory membrane much before the twelfth second. At this point, the arm ceases to increase in volume, and three seconds later it begins to decrease. At the twentieth second it shows a diminution of 1·5 cubic centimetres. The curve then slowly returns to the base line, which it almost touches at the thirty-eighth second. It indicates a second diminution of one cubic centimetre at the forty-fifth second. After this the curve rises slowly and crosses the base line at the sixtieth second. This is followed at intervals of about ten seconds by three positive waves and one negative wave, not shown in the figure, of somewhat less than five cubic centimetres. This curve would seem to indicate that the odour of isobutyric acid produces a reflex stimulation of the vasoconstrictor fibres of the arm, but I do not think it furnishes sufficient evidence for such a conclusion. The curve in other places

frequently shows just the opposite effect, although the conditions remained the same. To be at all certain of the effect of any particular stimulation we must, at least, know the general movement going on for some time before and after the stimulation. To avoid the inconvenient length of the original records needed for such a study, I have condensed their data into plotted curves, several of which (Plates IV, V, VI, and VII) accompany this article. In these curves the distance between the ordinates marks intervals of ten seconds each. Curve A, at the bottom of the plates, gives the changes in the amplitude of the pulse waves. The absolute value of this amplitude might easily be determined empirically, but this I have not done. Nor can I calculate it exactly, since I failed to keep a record of the magnification due to the leverage of the writing apparatus. But as this leverage was not varied during any of the individual records, its effect could have no influence on the changes which occur during the same record. The vertical distance between the abscissæ represents about two-tenths of a cubic centimetre in volume. Curve D gives the heart rate. For this curve the distance between each two abscissæ represents one beat per minute. Curve C gives the changes in the volume of the arm in cubic centimetres. The vertical distance between the abscissæ represents a change of two-tenths of a cubic centimetre. The pulse amplitude and the volume changes are calculated at every fifth second. The heart rate is reckoned for every ten or fifteen seconds. The time of stimulation by the various odours is indicated in the plates. The respiratory curve was taken in all the original records, and whenever it presented any marked irregularities, this fact is noted on the plates.

In order to study the effects of repetition and of individual variation in response to the same odours, the experiments were arranged in two series. The first series of experiments were all made on the same subject; the second series were made on twelve different individuals.

First Series.

Mr. George Bill, who acted as subject in the first series of experiments, is a mechanic, thirty-three years of age, in good health,

of fair muscular development, and not particularly susceptible to odours. His arm was kept in the cylinder about an hour daily from the middle of October to the middle of January. He was trained to keep his mind as blank as possible. The monotony of daily repetition soon removed the disturbances which in untrained subjects arise from the novelty of the surroundings. The odours of heliotrope, wood violet, wintergreen, musk, skatol, and indol were used daily; a few others were used occasionally.

Defects in the earlier forms of apparatus used render the experiments made during October and November unsatisfactory. Plates IV to VI, inclusive, give the results of three experiments of this series, which were made during December and January, when all the conditions were most favourable, and may serve as examples of the general results obtained from this series.

One of the most obvious features of these curves is the general increase in the volume of the arm which they exhibit during the course of the experiment. In one experiment there was an increase of 39 cubic centimetres in the volume of the arm between the 450th and the 2350th seconds; in another, an increase of 30 cubic centimetres between the 1st and the 1360th seconds; a third gave an increase of 35 cubic centimetres between the 20th and the 1300th seconds; and in a fourth the arm increased 37 cubic centimetres in volume between the 1st and the 1900th seconds.

Several facts indicate that this increase in the volume of the arm is due not to the temperature or pressure of the water in the arm cylinder, but to the mental condition of the subject; for in other experiments on the same subject, though the temperature and pressure of the water were the same as in the series just described, no such general increase in volume occurred, nor did it take place in experiments made on the other subjects. Second, the volume of the arm decreased immediately when anything attracted the subject's attention. In Experiment V (Plate IV) the subject was resting quietly with his arm in the cylinder for ten or fifteen minutes before I began to take the record. During this time the arm increased several cubic centimetres in volume. One hundred seconds

after the record was begun Dr. K. entered the room quietly, but was perceived by the subject. The volume of his arm decreased 11·5 cubic centimetres in the following 50 seconds. One hundred and fifty seconds later, as Mr. G. entered the room, a further decrease of 4 cubic centimetres in 20 seconds ensued. After both had left the room the arm immediately began to increase and continued to increase to the end of the experiment. In Experiment X, I spoke to him at the 240th second. This occasioned a fall of 5 cubic centimetres in a few seconds. Twice in the course of this experiment I turned on the odour of formic acid for a few seconds; each time it occasioned a sudden diminution in the volume of the arm.

The subject had grown familiar with his surroundings; the chair was very comfortable, the room quiet; there was nothing to attract his attention, and as a consequence his mind quieted down, and he was frequently dozing toward the end of the experiment. In Experiment VII he slept from about the 300th second to the end of the experiment. It was found, too, that in these experiments the heart rate became slower and steadier as the arm increased in volume—an indication that the subject was gradually quieting down or going to sleep. These facts, I think, show pretty clearly that this general increase in the volume of the arm is due to diminished mental activity; but, whatever may be its cause, it must be taken into account in calculating the effect of any particular sensory stimulation. To check an upward movement of the curve is practically the same thing as to cause a downward movement of a curve that is running horizontally. A horizontal line evidently can not be taken as a base line in calculating local changes. The base line must run in the general direction of the curve. It is not an easy matter to determine accurately what this line should be; but in most cases it may be approximated and a line obtained which will be much nearer the truth than a horizontal would be.

The curves also exhibit two species of local variation which do not seem to be connected with sensory stimulation. In most of the curves small oscillations occur, which suggest Traube-Hering waves. Besides these, larger and slower changes occur at irregular intervals,

which seem to be due to variations in the mental condition of the subject. These changes are the most annoying factors in the curves. I have been unable to eliminate them; and it is very difficult, if not impossible, to determine the part they play in the volume changes which take place during sensory stimulation.

Some peculiar features appear in Plate V, which are probably due to the fact that the subject was asleep during most of the experiment. The arm increased rapidly in volume from the beginning of the record to the 1300th second, and gradually decreased from this to the end of the experiment. The subject had been resting quietly with his arm in the cylinder for some time before the record was begun. He seemed to be asleep about the 300th second; at the 430th second I spoke to him and found that such was really the case. He did not wake, but his arm decreased 3 cubic centimetres in the following 20 seconds, and then increased more rapidly than before; 75 and 120 seconds later two other falls of about 4 cubic centimetres occurred which did not seem to be connected with any external stimulus. At the 1930th second, 50 seconds after a musk stimulation had ceased, there was a fall of 5·5 cubic centimetres in 30 seconds. At the 2250th second, during an indol stimulation, there was a fall of 6·6 cubic centimetres in 35 seconds, followed by an increase of 9 cubic centimetres in 265 seconds, when, 35 seconds after the indol stimulation had ceased, there followed another fall of 7·6 cubic centimetres in 30 seconds. The odour of wintergreen was now turned on for 150 seconds, during which time the volume of the arm increased 4 cubic centimetres; 5 seconds later there was a fall of 5 cubic centimetres in 35 seconds. During the following 400 seconds four or five similar falls of less extent occurred. At the 3150th second, during a skatol stimulation, the subject woke with a deep inspiration; the heart rate changed from 56 to 80; the volume of the arm increased 20 cubic centimetres in 70 seconds, and presently decreased almost as rapidly. He spoke to me at the 3250th second, and seemed asleep 100 seconds later. This sudden increase in the volume of the arm upon awaking is noteworthy, as ordinarily the reverse result is obtained, the arm decreasing in size as the subject returns to a con-

scious condition. The probable explanation of the unusual result in this case will be given presently in speaking of the changes in heart rate.

How are these changes in the volume of the arm to be interpreted? The curve throughout its entire extent exhibits rhythmic changes which suggest Traube-Hering waves. Are the larger changes mentioned above of a similar nature? There is a certain periodicity about them, and, if we except the sudden increase in volume which occurred at the 3150th second, they resemble the small changes in all but extent, and, even in this respect, it will be seen that they gradually shade off into each other. It is possible that they are caused by variation in cerebral activity. This explanation would harmonise with Mosso's observations on the changes in cerebral circulation during sleep.* Changes in mental activity invariably cause changes in the volume of the arm; the play of the sleeper's imagination may account for the changes with which we are here dealing. Some facts in the curve itself point in this direction. The fall at the 430th second was apparently caused by my speaking to the subject. The large increase in the volume of the arm at the 3150th second upon awaking appears to be due chiefly to the very great acceleration in heart rate, which occurred at that moment. This was during a skatol stimulation, but it will be seen from the other records that skatol does not, itself, quicken the heart rate. Did the foul odour occasion an unpleasant dream in which some spasm of emotion caused the sudden increase in heart rate and woke the subject? Speculation concerning the origin of these changes, however, will be of little value until we have more data at our disposal. The question which immediately concerns us here is whether or not, when all due allowances are made for changes produced by other causes, the curve still presents any evidence of sensory reactions. We will be in a better position to answer this question after we have made a comparative study of the other curves. The odour of formic acid was administered once for a few seconds in Experiment IX and twice in Experiments

* Mosso. Kreislauf des Blutes, Leipzig, 1881, S. 74 ff.

VI, VIII (Plate VI), and X. Each time it prevented full respiration, and caused a sudden and quite large decrease in the volume of the arm. The same result was invariably obtained whenever I used formic acid. Lehmann * obtained similar results with ammonia and bisulphide of carbon, which he used as typical unpleasant odours. I think, however, that the reactions in these cases are due in greater measure to the irritating action of these substances on the terminations of branches of the trigeminal nerve in the mucous membrane of the nose than to their odours.

In Experiment VIII, Plate VI, acetic acid was administered at the 1260th and again at the 1410th second. Each time it produced a diminution of 9 cubic centimetres in about 50 seconds. It was also administered in Experiment VI at the 1400th second, in Experiment X at the 1450th second, and at the end of Experiment XI; but in none of these cases did it produce any marked reaction. The cause of this discrepancy in results is not apparent.

Propionic, butyric, isobutyric, and valeric acids were administered on several occasions without any very decisive reactions appearing.

In Experiment VI propionic acid was administered at the 2225th second. A fall of 4 cubic centimetres occurred during the 65 seconds of the stimulation; but it is not at all certain that this fall was due to the acid, since similar falls occur throughout this curve without any apparent dependence on sensory stimulation. In Experiment VIII, Plate VI, at the 2160th second the volume of the arm increased 3 cubic centimetres during the first 40 seconds of a propionic acid stimulation, and decreased 1·8 cubic centimetres during the remaining 20 seconds. But this stimulation followed immediately upon a fall of 11 cubic centimetres produced by formic acid. The curve usually rises in this manner in such cases without any sensory stimulation. The fall during the latter part of the stimulation was possibly due to the acid. Propionic acid was administered again at the 1870th second in Experiment X. There was a rise here of 1·5

* *Op. cit.*, p. 84.

cubic centimetres during the 20 seconds of the stimulation; but this rise is in the general direction of the curve, and presents no evidence whatever of a sensory reaction. In Experiment VI butyric acid was administered from the 1710th to the 1890th second, and valeric acid from the 2000th to the 2175th second. Falls occurred in both instances; but from the general movement of the curve it would appear that they are rhythmic contractions, and independent of the stimulations. At the 1920th second in Experiment VIII, Plate VI, the curve was rising rapidly and continued to rise, apparently uninfluenced by the butyric acid which was turned on during the following 50 seconds. At the end of the stimulation the curve took a horizontal direction, which it kept during the following 130 seconds. Valeric acid was administered during 75 seconds of this time without producing any apparent effect. Butyric acid was also administered at the 1715th second in Experiment X. A fall of 2 cubic centimetres occurred during the first 30 seconds, but the curve shows that this also may be a rhythmic contraction. Isobutyric acid was used once in each of the Experiments VI, VIII, IX, X, and XI without any apparent effect.

Besides formic acid and the two instances of acetic acid mentioned above, there are three other causes which, when present, usually produce a marked diminution in the volume of the arm. These are a deep inspiration, muscular movement, and mental activity. A typical effect of a deep inspiration occurred at the 1070th second in Experiment X. This diminution in the volume of the arm seems to be due in part to the increased negative pressure in the pleural cavity, but I think it probable that a factor in the production of this reaction is the change in the mental condition of the subject, which usually accompanies an inspiration of this kind; for on such occasions the subject is usually drowsy, and with the deep inspiration the mind brightens temporarily. It would seem that there is a constriction of the peripheral arteries, causing an increased flow of blood to the brain. When a deep inspiration occurs while the mind is bright there is seldom any very large decrease in the volume of the arm. It is possible that when the change takes place during the

odour stimulation, the stimulation also plays a part in its production; but if so, the effect of the odour can not well be isolated. It is better, therefore, for present purposes, to discard such reactions. At the 2940th second in Experiment VI, I requested the subject to move his foot, which he did during the following fifty seconds. The heart beat immediately changed from 72 to 92, and after thirty seconds gradually returned to its former rate. This sudden increase in heart rate caused a transitory increase of 6·5 cubic centimetres in the volume of the arm, followed by a decrease of 13 cubic centimetres in fifty seconds. In this case it is probable that the effect was due in part to the muscular work and in part to increased mental activity. Whenever the subject moved his foot unconsciously, as he sometimes did, as at the 1670th second in this experiment, it produced a slight decrease in the volume of the arm, but of course there was not nearly the same amount of muscular work in these cases as in the instance referred to above.

The curves furnish numerous illustrations of the effect of mental work. This factor always causes a decrease in the volume of the arm. A typical instance of this occurs at the 100th second in Experiment V, Plate IV. Sometimes the fall is preceded by a transitory rise, as at the 1690th and 2940th seconds in Experiment VI; but in all such cases the rise is evidently due to a sudden acceleration of heart rate. Besides these changes, which can be definitely accounted for, the records of this series of experiments show a number of other variations in the volume of the arm. The general movement of the curve has already been discussed. In all the curves small oscillations occur every few seconds which are very much more pronounced in some records than in others, but which remain pretty much the same throughout the entire extent of the same record. These facts indicate that they are influenced by the condition of the subject rather than by any particular stimulation.

When due allowance is made for all these changes, do the records of this series of experiments present any evidence of pure odour reactions?

White heliotrope, wood violet, wintergreen, and musk were

chosen as specimens of pleasant odours; skatol and indol as unpleasant odours. The subject enjoyed the first four very much and disliked the latter two.

Heliotrope was administered at the 1365th second in Experiment V, Plate IV; at the 220th and 270th seconds in Experiment VI; at the 210th and 2800th seconds in Experiment VII, Plate V; at the 45th second in Experiment VIII, Plate VI; at the 850th and 1910th seconds in Experiment XI. In none of these instances is there any clear evidence of a reaction. There is not one feature common to these various curves. There is scarcely a resemblance between any two of them. The only approach to a resemblance among them is the upward tendency of the curve, but this is in the general direction of the curve, and evidently is not due to the stimulation.

What has here been said of heliotrope applies with equal force to the other five odours mentioned. Violet was administered at the 430th second in Experiment VI. The volume of the arm increased 2 cubic centimetres in the first thirty seconds and decreased 4·5 cubic centimetres in the following thirty seconds; it then increased to the end of the stimulation. Indol was administered at the 920th second in the same experiment, with very similar results. At first sight these look like odour reactions. But it was found that five other similar falls occur in the first 1,000 seconds of this experiment, at approximately equal intervals of time. The first two falls occurred before any odour was administered; the third, fifth, and sixth in intervals between stimulations. The latter portion of the curve presents a similar series of falls. Besides, if the falls occurring during the violet and indol stimulations were odour reactions, we would expect that they would be repeated with some regularity in numerous experiments on the same subject; but such is not the case. It is altogether probable, therefore, that we are here dealing not with sensory reactions, but with rhythmic contractions of the blood vessels. A comparison of the curves obtained by repeated applications of any one of these six odours will make it clear that, if the stimulation had any effect on the volume of the arm, it is very

thoroughly masked by other movements of the curve. If the odours really produced any characteristic effects they would come out in a composite curve made from a large number of experiments. I have not yet a sufficient number of experiments to give any value to such a composite curve.

The experiments presented here are essentially similar to a number of other experiments made on the same subject, all of which were conducted with great care; but the results obtained do not justify any such conclusions as those which Lehmann deduces from his experiments.

"Jeder lusterregende Eindruck erzeugt eine Vergrösserung des Volumens des Armes und der Höhe der einzelnen Pulsschläge nebst einer Vergrösserung der Tiefe des Atemholens." *

"Einfache, unlusterregende Sinneseindrücke rufen, wenn sie schwach sind, sogleich eine Verminderung des Armvolumens und der Höhe der einzelnen Pulsschläge hervor. Das Volumen nimmt bald wieder zu, trotz der Verkleinerung der Pulsschläge, und überschreitet gewöhnlich die Norm, wenn die Pulsschläge ihre vorige Grösse erreicht haben, die übrigens im allgemeinen ebenfalls überschritten wird. Bei stärkeren, aber doch nicht schmerzhaften Eindrücken treten diese Veränderungen mehr hervor und werden zugleich unmittelbar nach Anfang der Reizung von einigen tiefen Atembewegungen begleitet." †

"Einfache lustbetonte Sinnesempfindungen werden von einer Gefässerweiterung begleitet und vielleicht auch zugleich von einer Vergrösserung des Umfanges der Herzkontraktionen in Verbindung mit einer Erhöhung der Innervation der willkürlichen Muskeln, jedenfalls der Atmungsmuskeln." ‡

An inspection of the curves of this series will satisfy any one that none of these statements is true of them. Heliotrope and wood violet are certainly pleasant odours and were enjoyed by the subject; yet the volume of the arm diminished quite as often as it increased during their application. Indol and skatol are unpleasant odours, yet

* *Op. cit.*, p. 82. † *Ibid.*, p. 89. ‡ *Ibid.*

the volume of the arm frequently increased during the first few seconds of their application, and then decreased. It is not certain, however, that any of these changes were pure odour effects. It is but proper to say that the results obtained in this series with the perfected apparatus were all obtained from the subject after he had been repeatedly used in similar experiments involving the same set of odours. There were some indications that the vasomotor effects in the beginning were more pronounced than toward the end, when the odours had lost somewhat of their pleasant or unpleasant effects and the subject endured them in a rather perfunctory manner, but it is not possible to speak definitely as to this point, since the earlier apparatus employed was subject to so many errors that the records then obtained were not trustworthy.

Second Series.

The second series of experiments was made on twelve students, ranging from twenty to thirty years of age. Each experiment lasted about an hour. The same odours and acids were used as in the former experiments. There are some features common to all these experiments.

In the first couple of experiments with each subject the curve exhibited very little of the tendency to rise which constituted such a prominent feature of the series already described; but this tendency became more pronounced in the third and fourth experiments. Where the general tendency of the curve is upward the heart rate also becomes slower and steadier. This supports the view advanced above, that the gradual increase in the volume of the arm and the slowing of the heart rate are due to the diminished mental activity, and is therefore more pronounced when the subject has become accustomed to the plethysmograph and ceases to feel the mental excitement naturally attendant upon a novel experience. The small oscillations in the curves are, as in the former series, characteristic of the experiment. They are much more pronounced in some experiments than in others, but remain pretty constant throughout the whole extent of the same experiment.

The reactions in all the experiments are in the same direction, but they vary in extent with the different subjects, with the same subject at different times, and with the different stimuli.

Plate VII (Experiment XII) will serve as an illustration of the reactions obtained from subjects who are sensitive to odours. This was the third experiment on Mr. D., who is a student twenty-two years of age, of rather slight build, and nervous temperament.

The amplitude of the pulse wave diminishes to about one half at the first odour stimulation,* and does not again return to its original size. The heart rate slows up considerably during the experiment. The general tendency of the volume curve is slightly upward.

The subject was resting quietly with his arm in the cylinder for ten or fifteen minutes before the record was begun. During this time, and for the first 370 seconds of the record itself, the volume of the arm increased slowly, without exhibiting any very marked changes. Heliotrope was administered from the 370th to the 725th second. During the first 50 seconds of this period the volume of the arm decreased 19·5 cubic centimetres. It increased about 1 cubic centimetre during the following 50 seconds, and 14 cubic centimetres during the next 125 seconds. This was followed by a fall of 4·5 cubic centimetres in 25 seconds, and a rise of 7·5 cubic centimetres in 100 seconds. The prompt fall at the beginning of the stimulation, and the fatigue effect after about 100 seconds are pretty good evidence that this is an odour reaction, especially when it is taken in connection with the character of the curve previous to the stimulation. This conclusion is confirmed, I think, by the fact that a similar reaction was obtained every time I used this odour on Mr. D. Two other heliotrope stimulations occur in this record with results very similar in everything but extent; and this difference is at least partially accounted for by the fact that at the 740th second I moved the terminal plate farther from the subject's nose so as to diminish considerably the strength of the stimulation.

The fall of 18 cubic centimetres which occurred between the 740th and the 790th second is a mental reaction caused by my

* The original record of part of this stimulation, reduced in size, is given in Plate III, Fig. 1.

speaking to the subject and readjusting the odour plate. When the volume of the arm had returned to about its former condition and seemed inclined to remain pretty steady I turned on wood violet. It will be seen that the reaction is very similar to the previous heliotrope reaction, only less extensive. There is a fall of 8·4 cubic centimetres in the first 30 seconds. It remains down about 25 seconds, and increases 6·5 cubic centimetres in 70 seconds, when a second fall of 3·7 cubic centimetres occurs. Wintergreen was turned on from the 1265th to the 1440th second; the reaction is very similar to the previous violet reaction in every respect but the extent of the second fall. I had requested the subject before the experiment began to leave his mind as blank as possible, and not even to speculate about the nature of the odours. So great, however, was the down sweep of the curve that I suspected the presence of a mental reaction, and accordingly at the 1500th second I turned on a current of odourless air strong enough to be felt on his face. When the experiment was over I remarked to him that he had not obeyed my instructions, but had been indulging in speculations concerning the odours. He assured me that such was the case on only one occasion for a few seconds, when he felt the air coming from the tube but could perceive no odour. This indicates that the reaction in the other instances are connected in some way with the odours. At the 1650th second heliotrope was again administered. There was a prompt fall of 10 cubic centimetres in 35 seconds, followed by two quite large falls during the course of the stimulation. After an interval of 150 seconds, during which time there was very little change in the volume of the arm, musk was turned on. It occasioned a prompt fall of 8 cubic centimetres in 20 seconds. The two secondary falls were very slight. Skatol was turned on from the 2640th to the 2740th second without any apparent effect. Heliotrope was administered for the third time at the 2760th second. The reaction is essentially similar to the two former instances. I spoke to him at the 3060th second. An acquaintance entered at the 3150th second. When the subject had quieted down I turned on formic acid for 35 seconds. This caused a fall of 18 cubic centimetres in 50 seconds.

This record, as has already been said, was chosen as a specimen of very pronounced odour reactions, but each of the twelve subjects on whom this series of experiments was made gave unmistakable evidence of reactions to odours and irritant vapours. The reactions were always in the same direction, although seldom as pronounced as in the case of Mr. D. In the mental reactions obtained in this series it was found that the volume of the arm decreased very markedly notwithstanding a simultaneous quickening of the heart rate. This is characteristic of the mental reactions in all my experiments. A second striking peculiarity of the mental reactions is their long continuance. The effect of odours or irritant vapours passed off in three or four minutes at most, whereas mental work frequently kept the heart beating at a high rate and kept the volume of the arm low for many times that period.

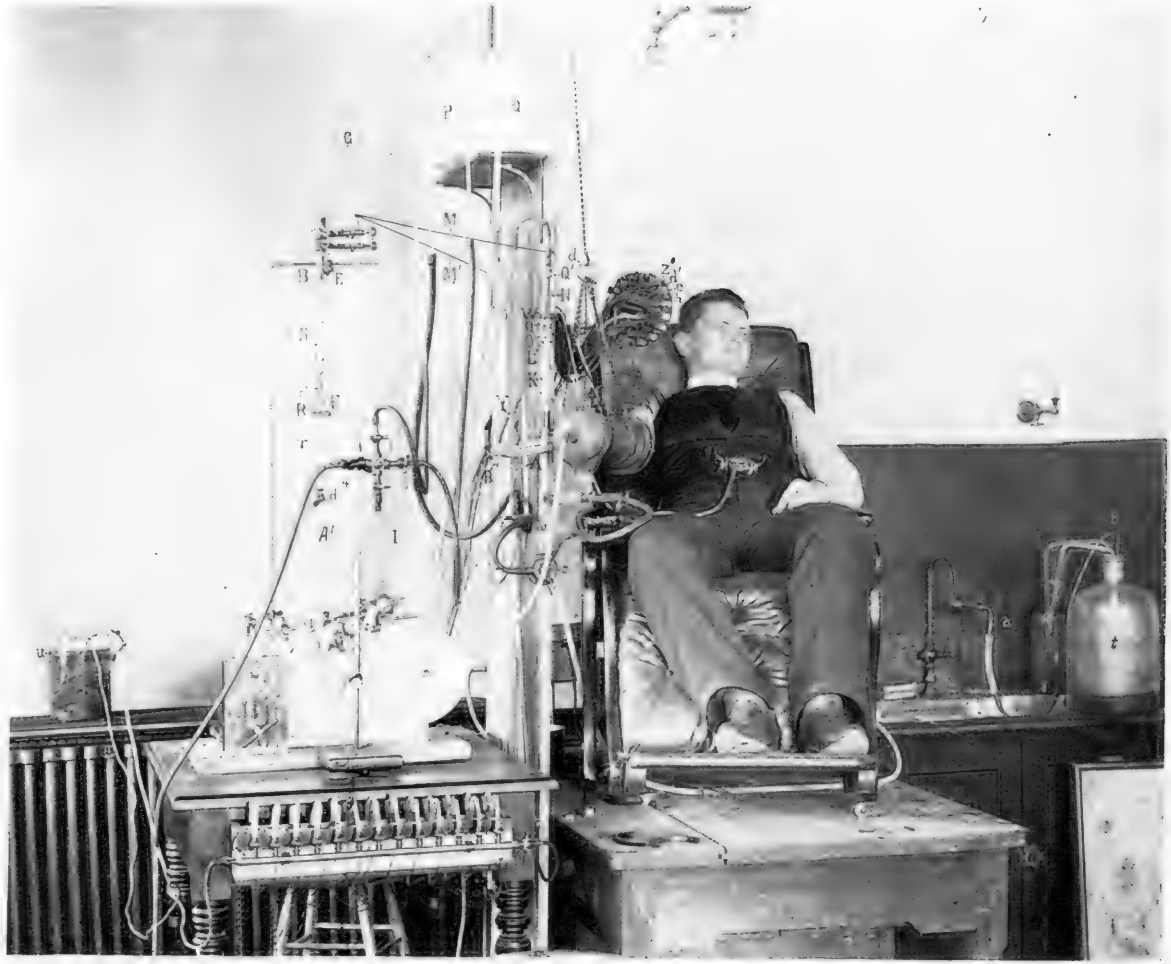
The relation existing between the effects of sensory stimulation and of mental work will be discussed in a future paper.

Summary.

The most important outcome of this investigation has been the completion of various improvements in the construction and use of the plethysmograph, by means of which numerous errors attending the use of the instrument have been eliminated.

The results of the work show that all olfactory sensations, so far as they produce any effect through the vasomotor system, tend to diminish the volume of the arm, and therefore presumably cause a congestion of the brain. Whenever the stimulation occasions an increase in the volume of the arm, as sometimes happens, it seems to be due to acceleration of the heart rate, which, of course, tends also to increase the supply of blood to the brain. The effect of odours varies in extent with different individuals, and with the same individual at different times. It was most marked in subjects sensitive to odours. Irritant vapours, such as formic acid, have a marked effect in the same direction—that is, they cause a strong diminution in the volume of the arm. The experiments give no support to the view that pleasant sensations are accompanied by a diminution of the

PLATE I.



blood supply to the brain and unpleasant sensations by the reverse effect. In all my experiments mental work caused a marked and prolonged diminution in the volume of the arm. This vasomotor effect was sometimes preceded by a transitory increase in the volume of the arm caused by acceleration of heart rate.

Acknowledgment.

I wish to return my sincere thanks to Prof. Howell, at whose suggestion this work was undertaken, and whose kind supervision and advice was a constant help and encouragement at every step of its progress. I am further indebted to him for his generosity in providing a subject for the first series of experiments. I am also indebted to Prof. E. A. Pace, of the Catholic University, for many valuable suggestions on the psychological aspects of the problem, and to the Rev. C. A. Ramm for suggestions in the constructions of the apparatus, and for the three accompanying drawings.

I must also take this opportunity of thanking the young gentlemen of St. Mary's Seminary, who generously placed their time at my disposal in acting as subjects for the second series of experiments. My thanks are due in a special manner to Messrs. Riedel and Beavan, who acted as subjects in a number of experiments and who assisted me in the laborious task of tabulating the results and plotting the curves.

June 1, 1895.

Explanation of Plates.

Plate I.—General Arrangement of Apparatus.

A, B, support for test tube and siphon; C, D, D′, clamps for same; E, clamp for spiral; F, F′, wires from spiral to rubber ring; H, I, tube connecting siphon with arm cylinder; J, four-way; 1, 2, 3, 4, stopcocks on same; K, piston tube; S, metal cap for same; M, M′, stay rods to upright; N, piston rod; O, O′, rods connecting metal cap with washer; P, Q, supply jars; Q, milled nut for adjusting level of piston; Q′, milled nut for adjusting indicator; R, rubber ring carrying test tube; S, spiral suspending test tube; T, test tube; U, upright; W, washer over milled nut on piston rod; X, tambour; Y, T-way in tube connecting piston tube with tambour; A, B, stopcocks on same; *w*, Z, three-way stopcocks on supply tubes; A, aspirator; *t*, bottle for washing air; U, bath for warming air; *y*, air tube on face of kymograph drawer; *z*′, terminal plate; *e*, electro-magnet; *d*′, armature; *h*, binding screw; *d*, ring suspending arm cylinder.

PLATE II.—ARRANGEMENT OF CYLINDER AND METHOD OF HOLDING THE ARM RIGID.

A, arm ring; B, wristlet band; C, D, rubber rings for making arm holder rigid to arm cylinder; G, H, faces of arm ring; M, M′, rods making arm ring rigid to wristlet; R, ring cemented into rubber sleeve; S, rubber sleeve; *e*, *l*, *m*, orifices in arm cylinder; *h*, thermometer; *t*, stopcock to let air escape from arm cylinder; Y, metal band for supporting cylinder; K, supply tube; *d*, ring from which arm cylinder is suspended.

PLATE III.—SPECIMENS OF TRACING (REDUCED ONE HALF IN SIZE) TAKEN UPON THE KYMOGRAPH.

1. Heliotrope stimulation (subject, Mr. D.). 2. Formic-acid stimulation (very light current of vapour). 3. Isobutyric-acid stimulation (subject, George Bill): A, signal—isobutyric acid turned on from 10th to end of record; B, respiratory curve—expiratory phase from 10th to 12th second shows that the stimulation could not have reached olfactory membrane before the 12th second; C, vasomotor curve; shows increase of 12 cubic centimetres in volume of arm between the 1st and 12th second, a decrease of 15 cubic centimetres at the 20th second, a return to base line at 38th second and a second decrease of 1 cubic centimetre at 45th second, and a return to base line at 60th second; D, heart rate. It shows 19 heart beats in first 15 seconds, and about 18 in each succeeding 15 seconds; E, time in seconds; F, base line; ordinates are drawn at every fifth second.

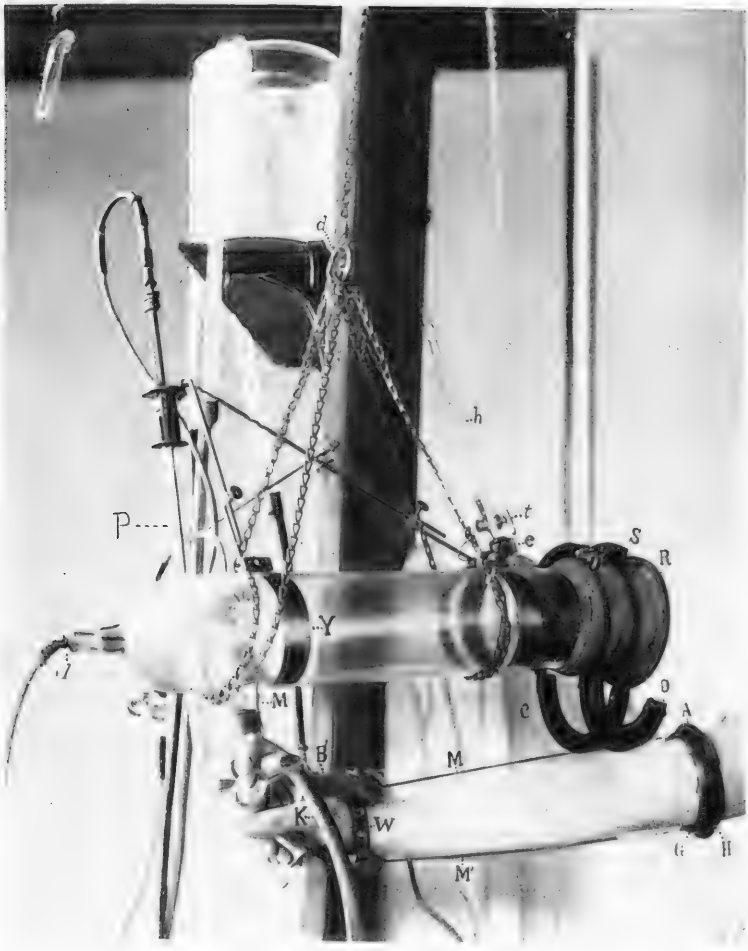
EXPLANATION OF PLOTTED CURVES. PLATES IV, V, VI, and VII.

A, changes in amplitude of pulse wave; vertical distance between abscissæ 0·2 cubic centimetre. D, heart rate; vertical distance between each two abscissæ represents one beat per minute. C, changes in volume of arm; vertical distance between each two abscissæ represents 0·2 cubic centimetre. The distance between the ordinates makes intervals of 10 seconds each. The pulse amplitude and volume changes are calculated at every fifth second. The heart rate is reckoned for every 10 or 15 seconds. The respiratory curve was taken in all the original records. Whenever it presents any marked irregularities this fact is noted on the plates. Time of stimulation by the various odours is indicated on the plates.

PLATE IV.—December 3d (subject, Mr. George Bill). Resting with arm in cylinder 15 minutes before record began. Volume of arm increasing slowly. Heart rate, 60. 100th second, Dr. K. entered. Volume of arm decreased 11·5 cubic centimetres in 50 seconds. 250th second Mr. G. entered. Volume of arm decreased 4 cubic centimetres in 20 seconds. Heart rate changed to 68, 64, 71, 64, 72, 68. 490th second, Dr. K. and Mr. G. leave room. Heart rate, 60. Volume of arm increases rapidly to 700th second, and more slowly to end of record. Subject dozing toward end of experiment. Heart rate gradually sinks to 52 at end of experiment. Total increase of volume of arm between 450th second and end of experiment, 39 cubic centimetres, due to diminished mental activity.

PLATE V.—December 12th (subject, Mr. George Bill). Resting quietly with arm in cylinder for some time before record began. Asleep from 300th second to end of experiment. Heart rate 60 during most of the experiment. No odour reaction. Volume of arm increases 30 cubic centimetres between 1st and 1360th seconds. Small rhythmic contractions every 10 or 15 seconds. Spoke to him at the 430th second, which occasioned a fall of 3 cubic centimetres in 20 seconds. 530th second, fall of 4 cubic centimetres. 650th second, fall of 4 cubic centimetres. 1930th second, fall of 5·5 cubic centimetres in 30 seconds. 2250th second, fall of 6·6 cubic centimetres in 35 seconds. 2515th second, fall of 7·6 cubic centimetres in 30 seconds. 2710th second, fall of 5 cubic centimetres in 35 seconds. Four or five similar falls of less extent occur during the following 400 seconds. None of these falls seem to be due to odour stimulation. They are probably rhythmic con-

PLATE II.



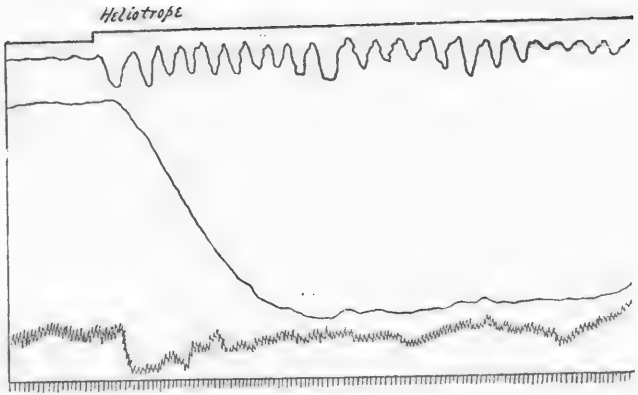


FIG. 1.

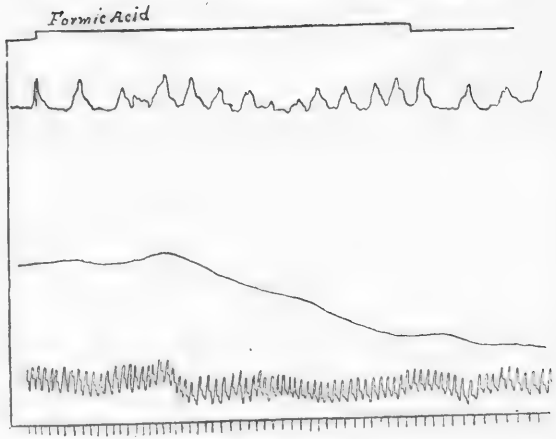
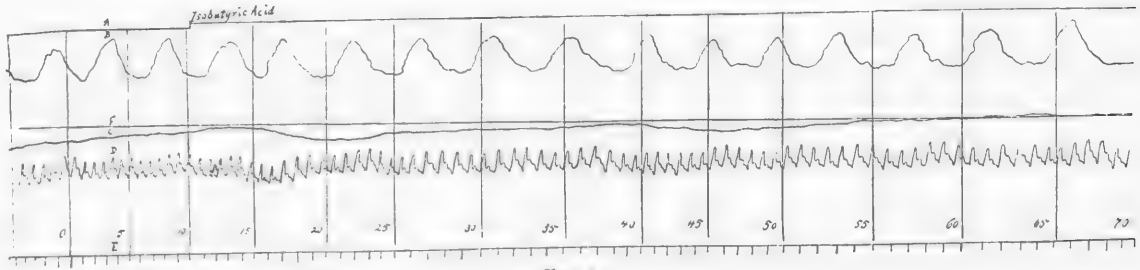
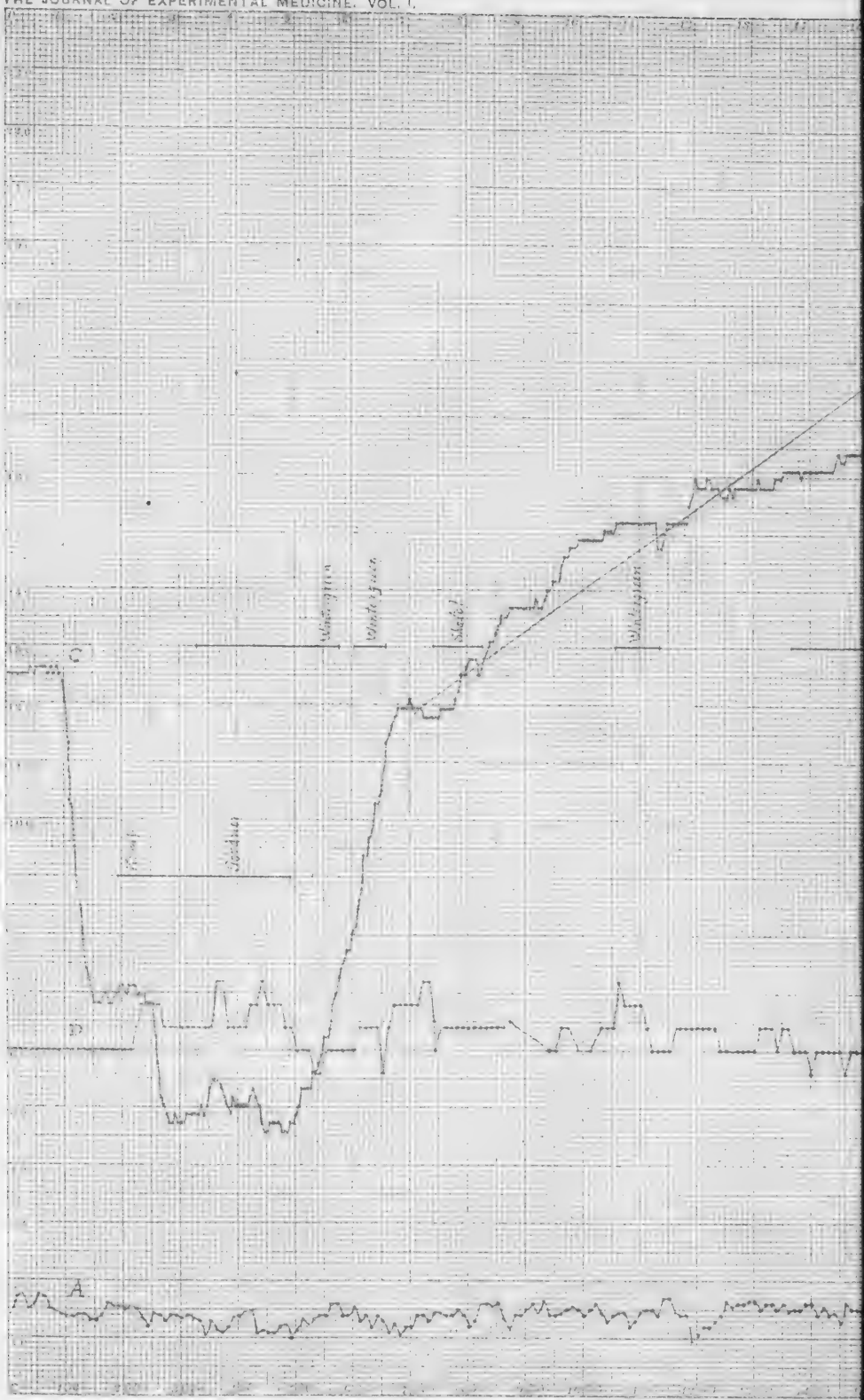


FIG. 2.

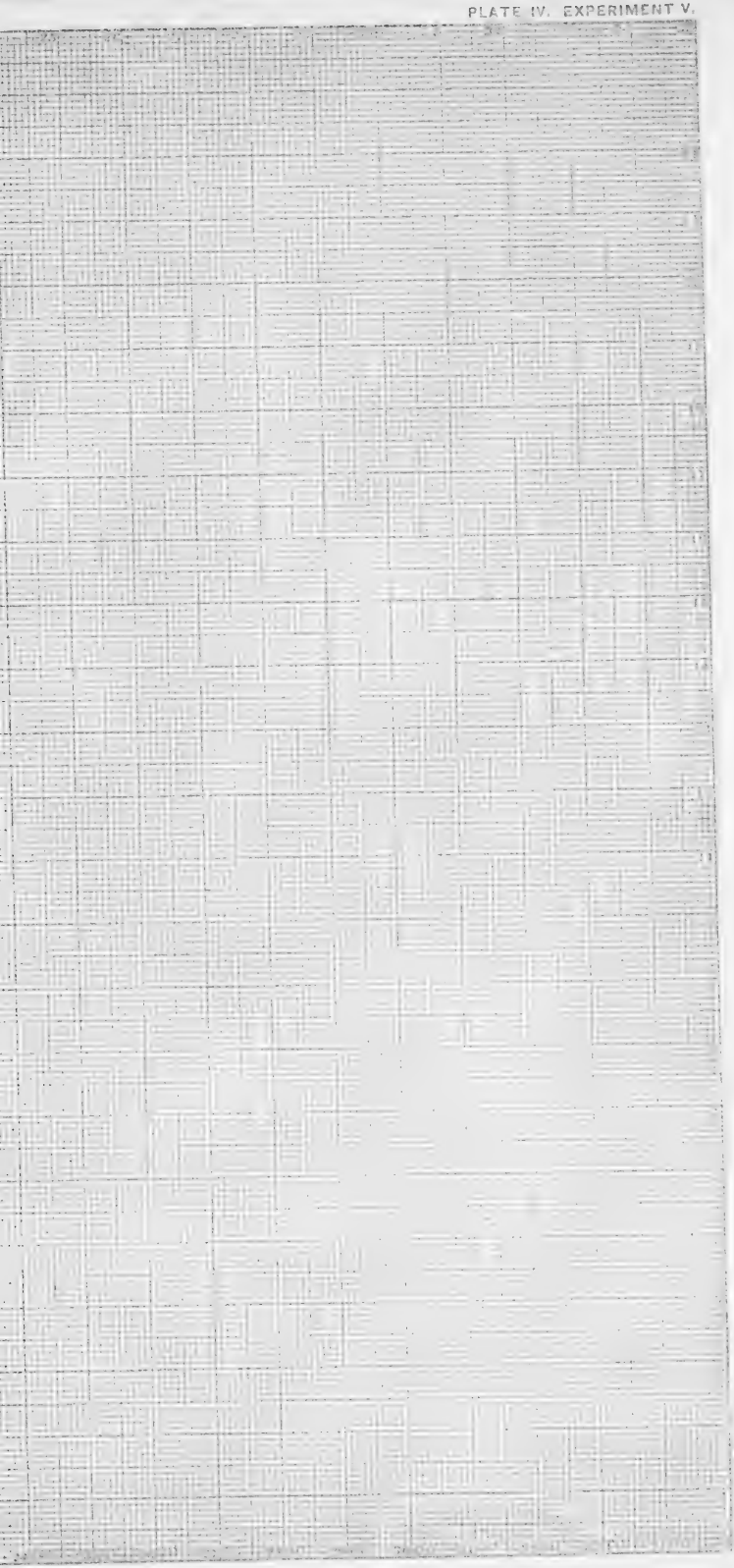


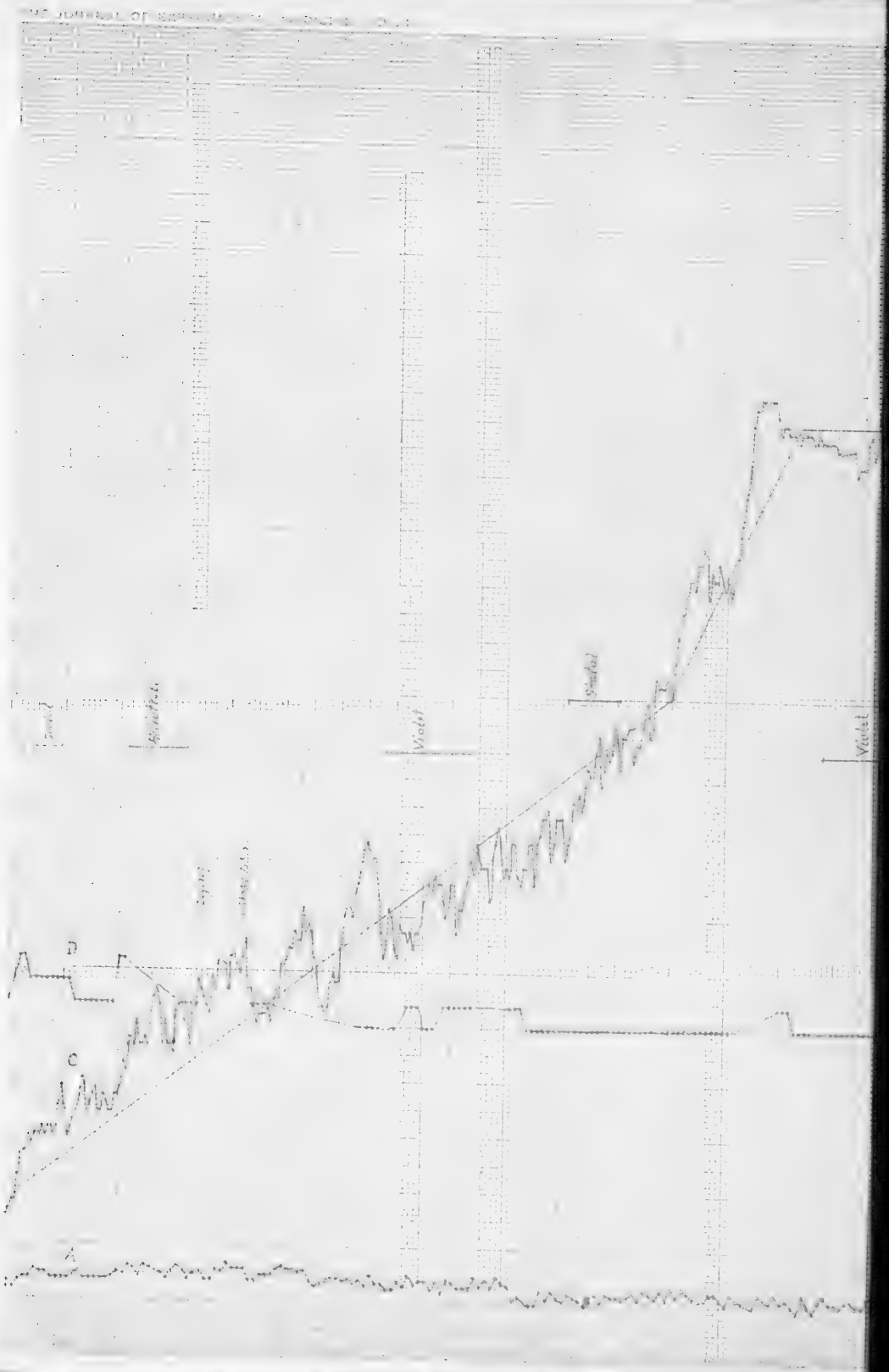
These tracings are reduced one half.

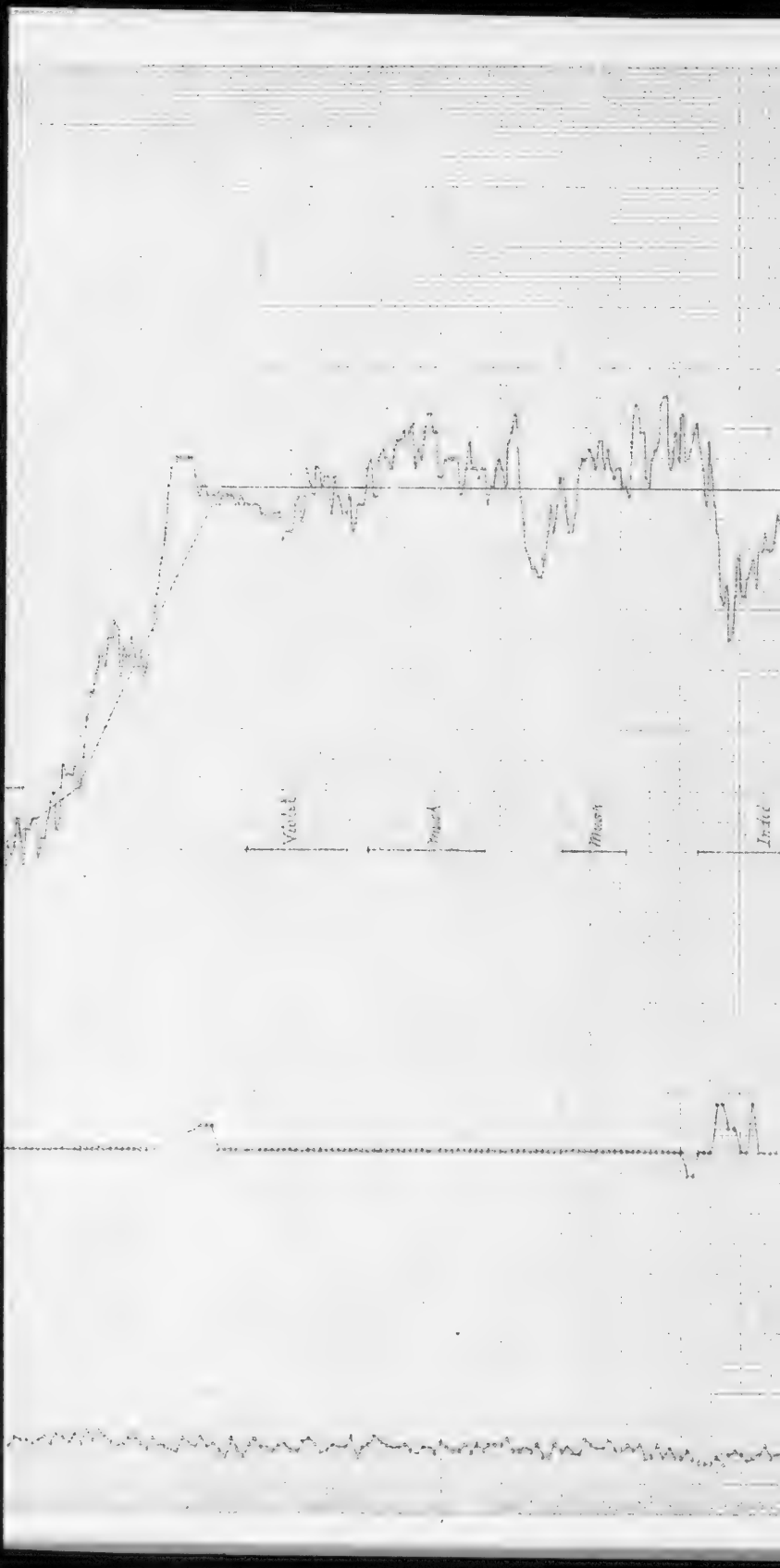
FIG 3.

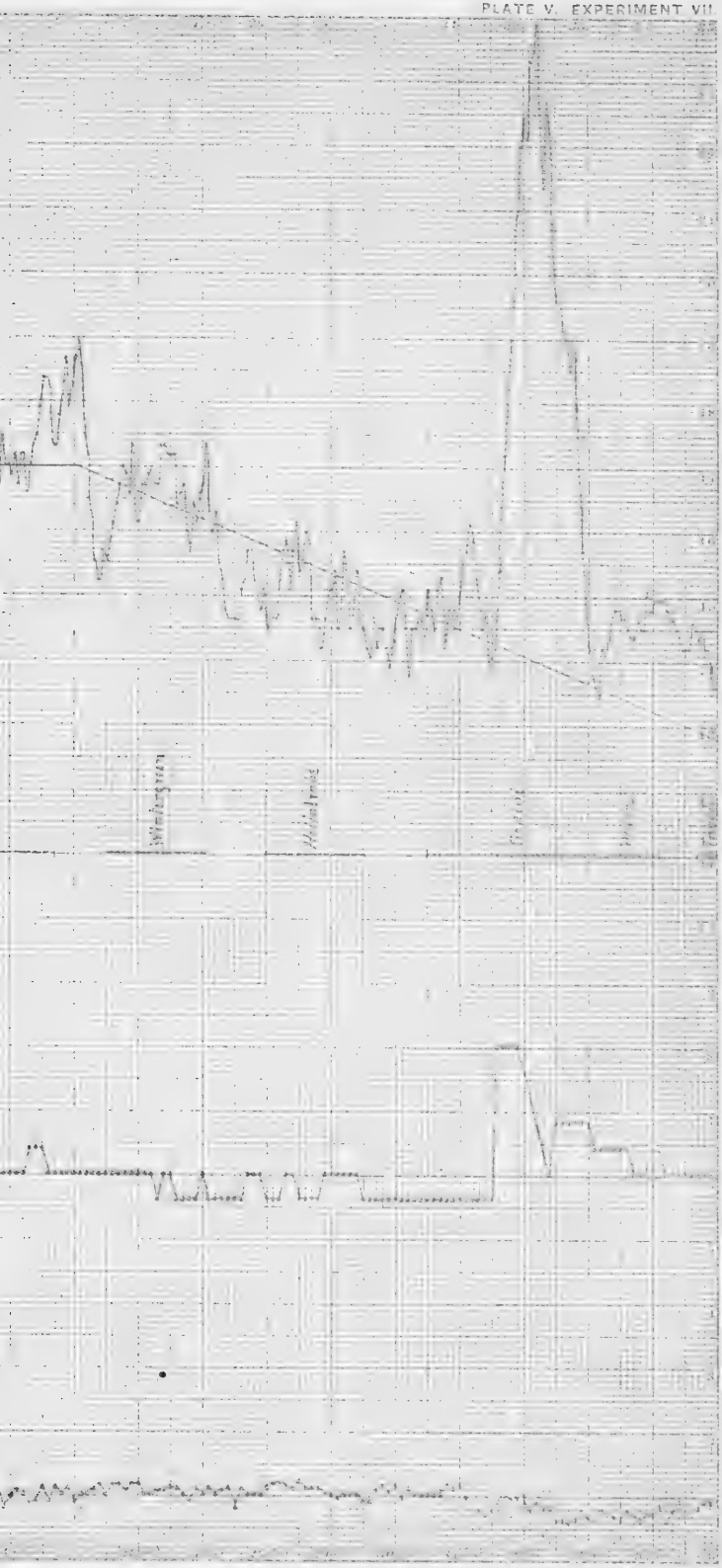

C
D
A

Violett
Violett
Violett
Violett

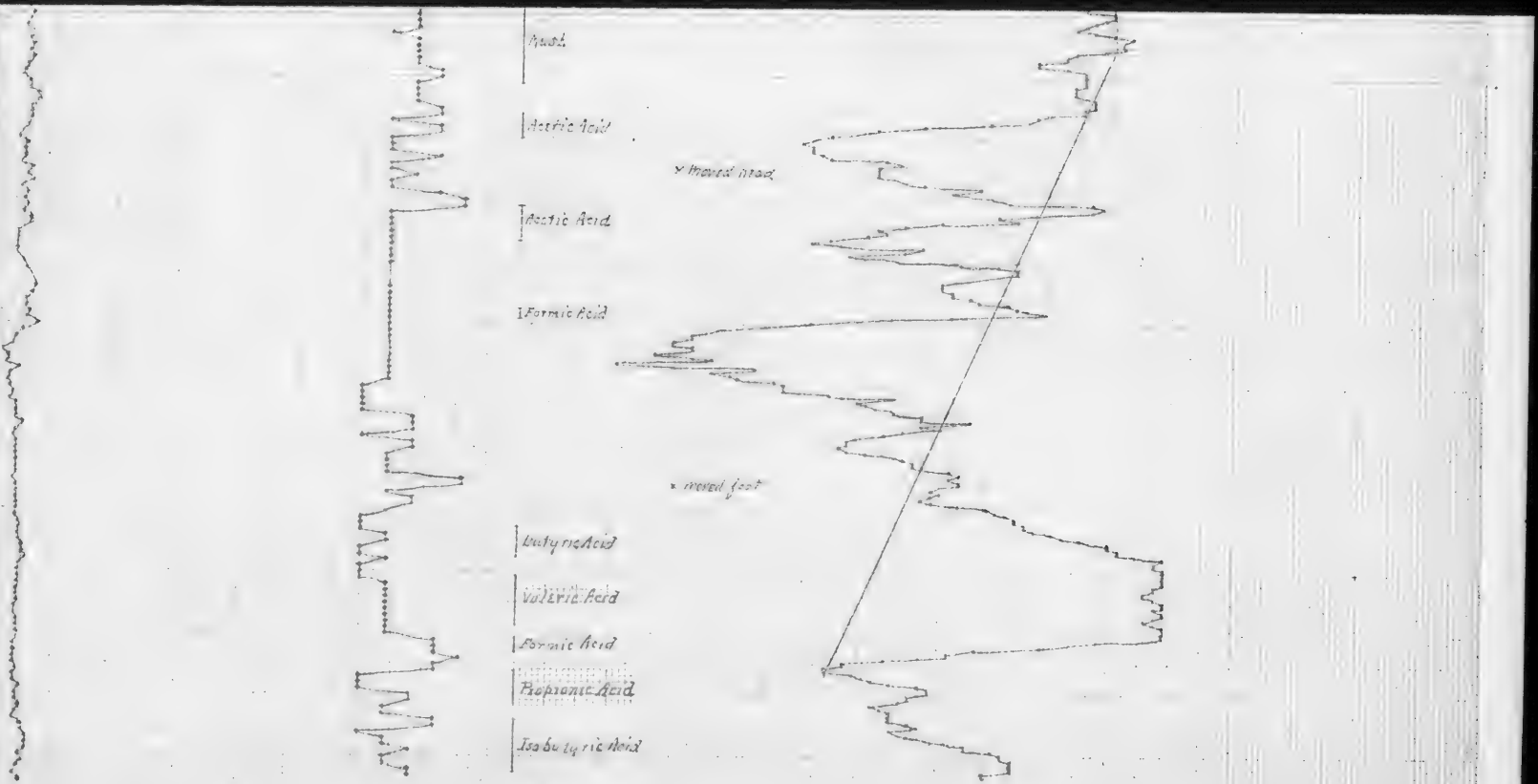


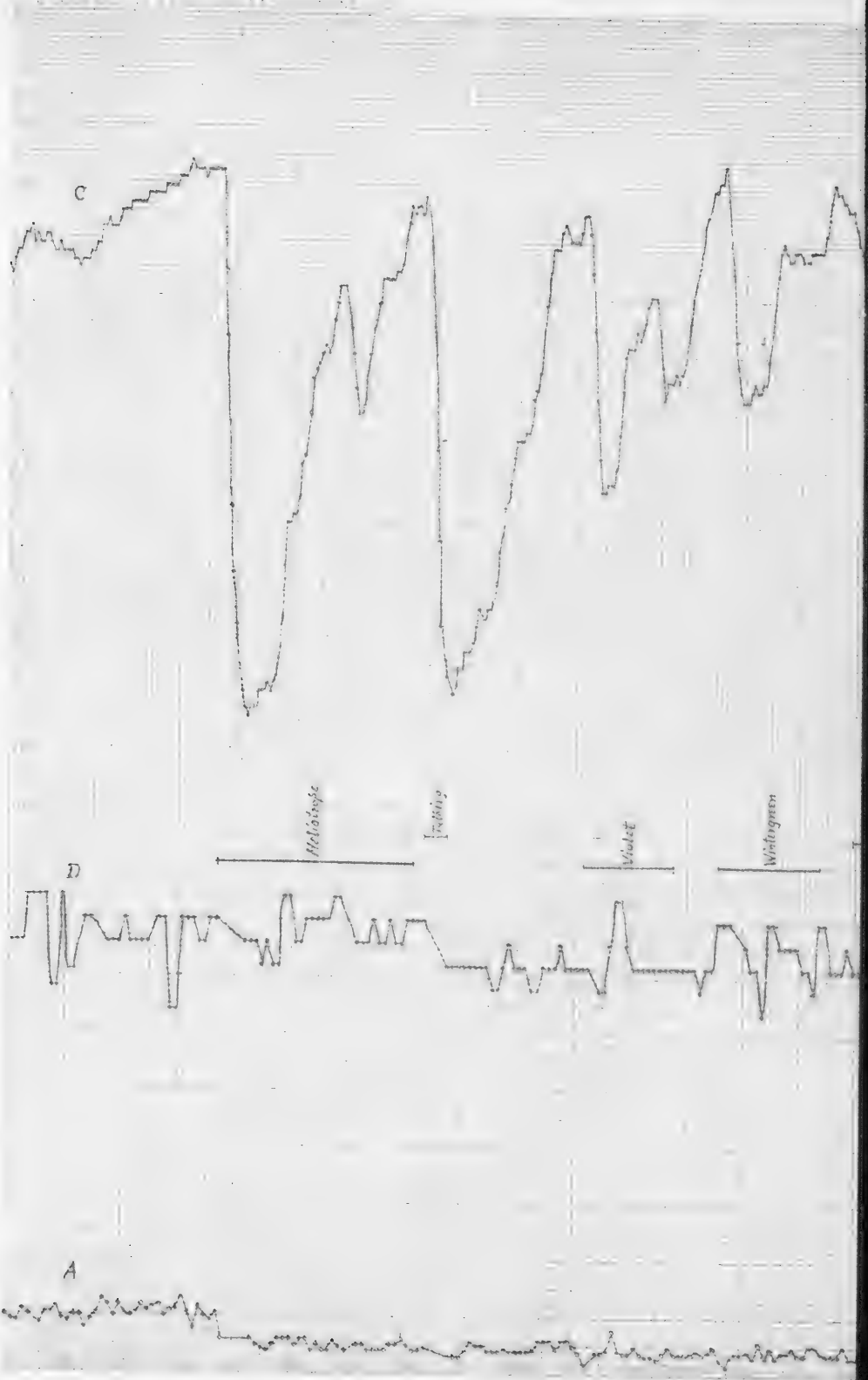

Violet
Violet
Violet
D
C
A


Violet
Blue
Green
Red



Indigo
Violet
Wintergreen
Scarlet
Indol
Musk
Musk
Acetic Acid
Acetic Acid
A


Acetic Acid
Formic Acid
Butyric Acid
Valeric Acid
Formic Acid
Propionic Acid
Isobutyric Acid


C
Heliotrope
Violet
Wintergreen
D
A

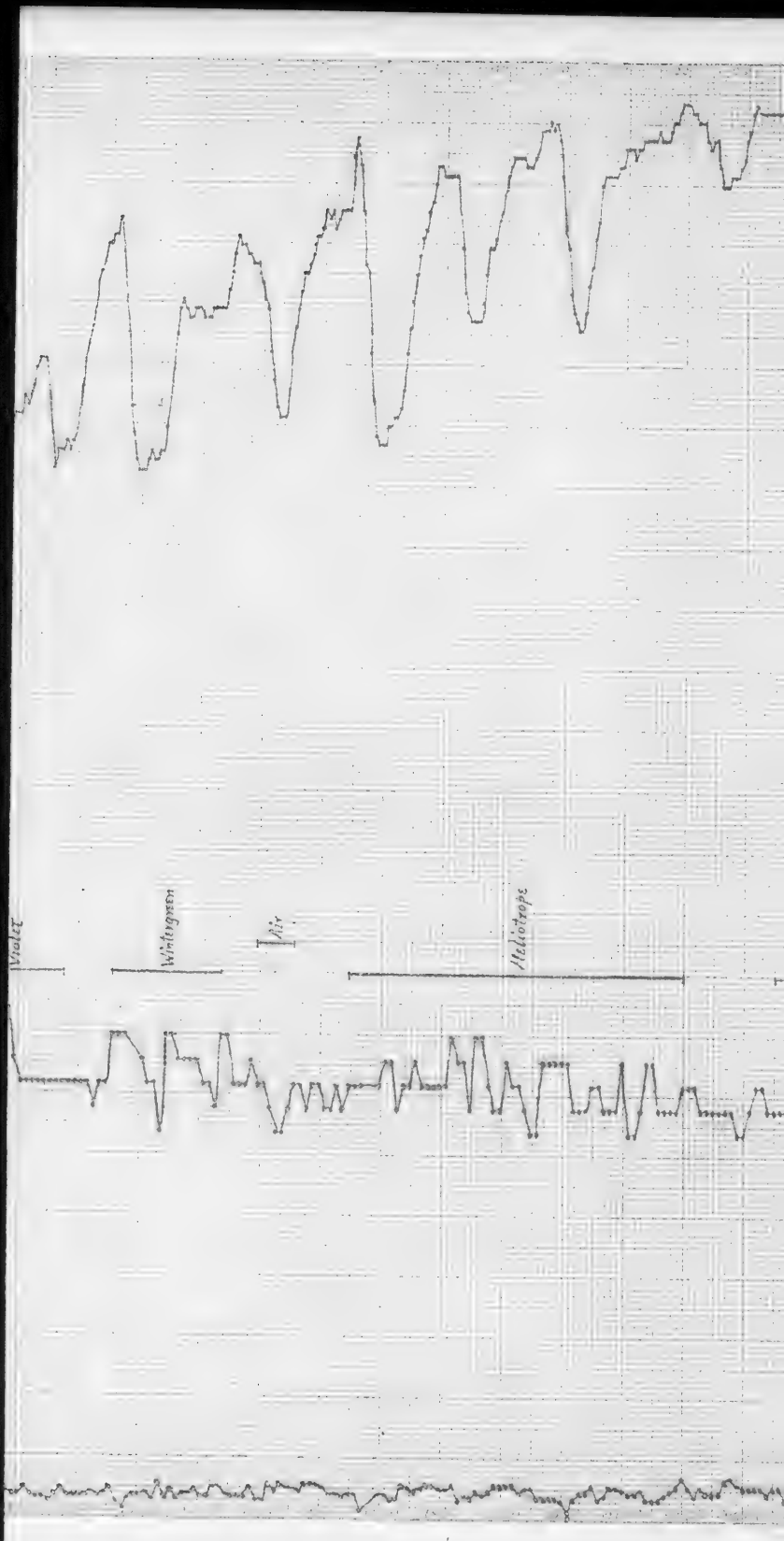

Violet
Wasserstoff
Luft
Heliotrope

PLATE VII. EXPERIMENT XII.

tractions. 3150th second, subject woke with deep inspiration. Heart rate changed from 56 to 80, probably due to a spasm of emotion caused by unpleasant dream. Volume of arm increased 20 cubic centimetres in 70 seconds, apparently due to quickened heart rate. 3250th second, subject spoke and seemed asleep 100 seconds later. Arm returned to its former volume and heart rate to 60. Amplitude of pulse wave diminishes from beginning to end of experiment. 3250th second, about one third its initial amplitude.

Plate VI.—December 14th (subject, Mr. George Bill). Volume of arm increases 12 cubic centimetres during first 1100 seconds. 1260th second, acetic-acid stimulation, diminution 9 cubic centimetres in volume of arm in 30 seconds. 1410th second, acetic acid, with similar result. 1580th second, formic-acid stimulation for 15 seconds, occasioning a fall of 13 cubic centimetres in 80 seconds. 2100th second, formic-acid stimulation for 30 seconds, with a fall of 11 cubic centimetres in 60 seconds. Odour stimulations apparently without effect throughout the curve. Amplitude of pulse wave diminishes gradually throughout the experiment.

Plate VII.—January 31st (subject, Mr. D). Subject resting quietly with arm in cylinder 15 minutes before record began, during which time and for the first 370 seconds of the record itself volume of arm increased slowly without any marked changes. Heart rate gradually slows during experiment. Volume of arm exhibits a slight upward tendency during most of the experiment. Amplitude of pulse wave diminished to about half at the first odour stimulation, and does not return to its original size. 370th second to 725th, heliotrope stimulation. Volume of arm diminishes 19·5 cubic centimetres in first 50 seconds, increases 1 cubic centimetre during the following 50 seconds, and 14 cubic centimetres during the next 125 seconds. 595th second, fall of 4·5 cubic centimetres in 25 seconds. 740th second, spoke to subject; fall of 18 cubic centimetres. 1020th second, wood-violet stimulation, fall of 8·4 cubic centimetres in first 30 seconds; second fall, 3·7 cubic centimetres at 1145th second. 1270th second, wintergreen stimulation, fall of 8·5 cubic centimetres in 30 seconds; a second slight fall at 1370th second. 1500th second, current of air; subject speculates as to its nature; fall of 4·5 cubic centimetres in a few seconds. 1650th second, heliotrope stimulation; fall of 10 cubic centimetres in 35 seconds, followed by two quite large falls in course of the stimulation. 2350th second, musk stimulation; fall of 8 cubic centimetres in 20 seconds, followed by two slight secondary falls. 2640th and 2740th seconds, skatol stimulation, no apparent effect. 2760th second, heliotrope; fall of 10 cubic centimetres in 30 seconds. 2870th second, fall of 2·5 cubic centimetres in 15 seconds. 2985th second, fall of 6 cubic centimetres in 30 seconds. 3060th second, subject spoke; fall of 11 cubic centimetres in 90 seconds. 3150th second, S. entered room; fall of 2·5 cubic centimetres in 30 seconds. 3270th second, fall of 3 cubic centimetres without obvious cause. 3450th second, formic-acid stimulation for 30 seconds; fall of 18 cubic centimetres in 50 seconds.

THE VASCULAR CHANGES OF TUBERCULOUS MENINGITIS, ESPECIALLY THE TUBERCULOUS ENDARTERITIS.

By LUDVIG HEKTOEN, M. D.,
Chicago.

PLATE VIII.

(*From Prof. Chiari's Pathological Institute in Prague.*)

The pathogenesis of tuberculous meningitis presents many questions of interest.

The favourite point of localization of the specific granulations of this frequent and intense process about the arteries, especially the Sylvian; the manner of primary invasion of the bacilli and of their secondary diffusion; the presence or absence of a mixed infection—these are some of the interesting problems that merit consideration.

In this article it is proposed to study somewhat in detail the histogenetic vascular changes in the leptomeninx in tuberculous inflammation, on account of their interest from the purely histological aspect and also of their possible significance as regards the mode of infection.

First it will be advisable to consider in general the various routes by which the infection may take place in tuberculous meningitis. Then it will be in order to introduce the more important statements in the literature concerning the arterial changes in the disease in question and their relation to the mode of infection. Upon this basis will be developed the results obtained by the bacteriological and histological examination of a number of instances of tuberculous meningitis, and also from certain, as yet unfinished, animal experiments.

The development of our knowledge concerning tuberculous meningitis is, naturally, very closely connected with that of tuberculosis in general.

The descriptions by Coindet* and Papavoine of the minute nodules characteristic of the tuberculous form of meningitis called attention forcibly to the question of the genesis of these peculiar elements. In the course of years the opinion gradually formed that the miliary granulations stood in some relation to the caseous masses nearly always found somewhere in the body of those dead from "water on the brain." Often tuberculous meningitis was, so to speak, only a part of a general miliary tuberculosis which Buhl, in 1872, characterized as an infectious and resorption disease, the miliary nodules bearing the same relation to the caseous foci as the metastatic abscesses of pyæmia to the primary pus focus.

Taking the accumulated facts into consideration, and especially the constant distribution of the tubercles along the pial vessels, the occasional limitation of the process to one definite arterial territory, together with the well-nigh constant presence of caseous masses somewhere in the body, Huguenin, writing in 1878, found the conclusion warranted that tuberculous meningitis usually originated from embolism by way of the blood of an unknown agent contained in, and most likely the cause of, the older tubercular lesions. This conclusion received material support by his finding perforation of a softened caseous focus into a pulmonary vein in an instance of general miliary tuberculosis with tuberculous meningitis.

Koch's discovery of the tubercle bacillus, and Weigert's conclusive anatomical demonstration of the routes of entrance from local areas of tubercle, subsequently furnished the direct and positive proof of what was generally believed to be the case from the circumstantial evidence mentioned.

The consideration of the mode of entrance of the tubercle bacillus in the meninges and the relation of tuberculous meningitis to tubercle elsewhere have, as already indicated, received much attention, and a large number of observations bearing on this point may be found in the clinical and statistical studies of Seitz, Henoch, Demme, Müller, Wortmann, Rieder, Medin, Reinhold, Northrup,

* The references to the literature are arranged at the end of the article and in alphabetical order, according to the names of the authors.

Klötsch, Neumann, Simon, and others. Suffice it to say that it is the general belief that in nearly all instances of tuberculous meningitis the invasion occurs by way of the blood, which may become infected, as shown by Weigert and others, from local tuberculosis, either directly from extension to the walls of veins or of large arteries, or indirectly through the lymph stream.

Often tuberculous meningitis is a terminal event in cases of extensive infection with the formation of multiple, chronic, and caseous foci and the diffuse eruption of miliary granulations in various organs, so that while it is perhaps no longer possible to trace any exact sequence of events, yet there can be no doubt of the originally hæmatogenous nature of the general infection. But many cases also occur in which the meningeal infection develops in connection with local circumscribed, often latent, foci in the lymph glands, the lungs, the pleura, the bones and joints, the skin, etc., and in these instances the blood is also held as the carrier of the bacilli, although a few authors attribute this rôle to the lymphatics in certain special cases. Babes thinks that when the peribronchial or retropharyngeal glands are especially involved, then the bacilli may reach the brain by retrograde transport in and along the lymphatics.* Hübbenet assumes the same route in one case of tuberculous meningitis occurring in connection with primary tuberculosis of the peribronchial lymph glands. Demme has an example of a probable lymphogenic pial infection from tuberculous rhinitis.†

Acute tuberculous meningitis following old foci in the brain, the spinal cord, and the skull or spinal column, is, of course, most likely due to a direct infection of the lymph spaces of the internal meninges. The inflammation, commencing in the vicinity of the point of origin, may become diffuse by dissemination of the bacilli through the cerebro-spinal lymph vessels (Ziegler); in addition a local dissemination through the arteries might also occur.

Concerning the occasional instances of so-called "spontaneous"

* The plexus jugularis profundus runs along the deeper vessels in the neck to the base of the brain, where it receives lymph from the cranial cavity.—Gegenbaur.

† The subarachnoid space communicates with the mucous membrane of the nose.—Stöhr.

or "primary" tuberculous meningitis, it can confidently be said that but very rarely, if at all, has the anatomical investigation been thorough enough to exclude all the primary hidden foci that may have been present as the origin of a hæmatogenous invasion. And yet the possibility of a primary meningeal tuberculosis (without the presence of an old focus) can not be denied. The demonstration by Straus of virulent tubercle bacilli in the nose of nine seemingly healthy persons out of twenty-seven examined, certainly warrants the suggestion that in some way tubercle bacilli might, under exceptional circumstances, such as after trauma (Demme, Coats*), be imported into the subarachnoid space, and thus cause a leptomeningitis in the same manner as appears to be the origin of many cases of non-metastatic cerebro-spinal meningitis.

Perhaps tubercle bacilli might also enter the pia from the middle ear, in which Kossel found them in a nursling.

Under all circumstances, the hæmatogenous origin of tuberculous meningitis (which is not the result of direct infection of the lymph vessels from chronic foci in the nervous system) must be regarded as the general rule, and all other possible modes of infection as exceptions of uncommon occurrence.

In accordance with the etiological considerations referred to in the preceding pages, the distribution of the tubercles along and upon the vessels in tuberculous meningitis is usually spoken of as a matter of course, as the direct result of the import of the bacilli by the blood; but, as may be seen from the usual descriptions of the morbid anatomy of this process, it can not be said that the exact relations of the vascular changes to the primary localization of the bacilli is very clearly understood. Indeed, it will very soon become clear that, notwithstanding significant isolated observations and also important special studies, such as those from Rindfleisch, Cornil, Guarnieri, and others, yet the changes in the arteries, as well as in

* Coats, in an instance of tuberculous meningitis developing after trauma, found tubercles in an organized dural clot. There were no tubercles in any other part of the body. The route of the infection is not discussed.

the veins, in tuberculous meningitis have in reality not been systematically studied in all their bearings.

It is well to begin the review of the literature concerning the arterial changes in tuberculous meningitis with one or two citations about arterial tuberculosis in general.

Orth states that until recently it was thought that the media and the intima were so immune from tuberculosis that they were never attacked either primarily or secondarily, but that newer investigations show that the intima may proliferate and form giant cells as well as tubercles independently of adventitial changes.

Ziegler teaches that tuberculous arteritis may come from infection by the blood, and that in tuberculous organs the arterial walls may be invaded by bacilli, giving rise to tubercle or to more diffuse inflammation and proliferation resulting in thickening of the wall that may end in caseation with thrombosis or rupture.

Among the first to study closely the histology of the miliary tubercles in the pia was Rindfleisch, who found the larger arteries the seat of a unilateral swelling, while the smaller presented spindle-shaped thickenings, all of which he believed were due to changes that originated in the adventitia.

Virchow writes that, generally speaking, it is easy to convince oneself that not the vessels, but the ground substance forms the matrix for the tubercles in the adventitia of smaller pial arteries.

Huguenin calls particular attention to his having observed that the miliary tubercles in tuberculous meningitis develop not only outside the blood vessels, but also from the endothelial lining of their interior, and that tubercles may perforate the vessel wall from within as well as from without.

Danforth, in 1878, concludes that the lesions peculiar to tuberculous meningitis are confined to the smaller blood vessels of the pia, and that they are always exterior to the tunica interna, and therefore have no structural relation to the circulating blood.

Lancereaux says the miliary granulations take birth in the lymphatic sheath of the blood vessels, most frequently at the

point of bifurcation, being produced by multiplication of the nuclei and the cells which enter into the composition of the adventitia.

Baumgarten agrees with Brodowski, Cornil, and Kiener that occasionally the endothelium may proliferate in tuberculosis of the arteries of the pia mater, but this is usually not extensive, and in fact an exception rather than a rule.

Arnold observes that he has found tubercles in the intima of larger vessels, and cites the similar observations of Ponfick in regard to the thoracic duct, of Weigert as to the pulmonary veins, and of Cornil in connection with the vessels of the pia, but concludes that it is impossible at the time of his writing to determine whether any of these vascular changes bear any relation to the spread of the tuberculous process or not.

Guarnieri studied the histological changes in twelve cases of tuberculous meningitis. Besides adventitial changes, the exudate, infiltration into nerves, and a hyaline degeneration of the vessel walls, he also demonstrated quite constant changes in the media and in the intima. The intima presented semilunar or circular accumulations of round and epithelioid cells in a granular substance between the endothelium and the elastic lamina so that the lumen was more or less narrowed. He found no giant cells in this proliferation, and but few tubercle bacilli compared to the number outside the arteries. This endarteritis, present also in the spinal pia and in the interior of the brain, was always accompanied with an older periarteritis and mesarteritis, the muscle fibres disappearing from the infiltration of round and epithelioid cells which frequently formed accumulations outside of the elastic layer. Guarnieri regards the changes as going from without inward.

Cornil, in 1880, found not only the usual adventitial changes, but also special changes in the intima which he characterized as an endarteritic or endophlebitic tuberculosis. Between the endothelium and the media of the arteries was a series of layers of voluminous cells, flattened by mutual compression, and among them cells with two to four nuclei as well as veritable giant cells with fifteen to

twenty nuclei. There was no marked production of limited nodules, but rather a uniform proliferation.

Cornil and Ranvier reproduce this description with the appropriate drawings in their *Manuel d'Histologie* of 1881.

Cornil and Babes describe, in addition to the tuberculous endarteritis of Cornil, the presence of tubercle bacilli in the endarteritic granulations, in the thickened and caseous walls, and in the interior of thrombosed arteries and veins, and they give several figures illustrating these observations.

While Cornil and Babes lay much stress on the active part taken by the vessels in the histological changes, yet they do not emphasize the relation of such changes to the route by which the bacillus enters the leptomeninx. In another communication they express the opinion that the spread of the bacilli through the blood is proved by finding them in or near the vessels.

Huettenbrenner, in examining the brain of a boy who died from basilar meningitis, found, among other changes, a typical tubercle with giant cells and caseation in the intima of a basal artery. The tubercle was located between the endothelium and the media, there was no oblique perforation of the vessel, and Huettenbrenner regards this as a primary intimal tuberculosis from direct implantation of the bacilli.

Rindfleisch teaches (in the last edition of his text-book) that the secondary localization of the tubercle bacillus which leads to a disseminated or general miliary tuberculosis occurs upon the smaller vessels, and that the classical place for studying the resulting tuberculous vasculitis is the basal arterial system of the brain, especially the sheath of the Sylvian arteries, whose adventitia will show the miliary granulations. In these cases the internal coats become secondarily involved and infiltrated with tubercular proliferation. On cutting open such a vessel longitudinally, one finds, in place of the familiar folds in the intima and the transverse striation of the muscular fibres, a sharply circumscribed defect occupied by tuberculous granulation tissue.

[3]Rindfleisch has repeatedly found places in which it appeared to

him as though the epithelioid cells originated from the muscular fibres and not from emigrated leucocytes, and he has a figure entitled "Muscular fibres which appear to be changing into tubercle cells." In other words, Rindfleisch regards the secondary localization of the bacilli as occurring in the adventitia of the vessels.

Birch-Hirschfeld states that the bacilli are assumed to reach the pia through embolism, and then to spread diffusely through the lymph spaces. The vessels certainly partake in the inflammation, but the changes consist rather in a diffuse cell infiltration than in the formation of tuberculous nodules.

Hirschberg studied three cases of tuberculous meningitis which presented focal symptoms before the appearance of the usual clinical manifestations. In two of these cases there was an endarteritis as well as an endophlebitis, with tubercle bacilli, giant cells, and caseation. The vascular process, older than that near by, is consequently regarded as the immediate source of the pial infection, the blood carrying the bacilli from the endarteritic focus. Hirschberg did not find any point at which the process had broken through the endothelial lining which covered the intimal proliferation; nevertheless, this must have occurred, because in one case the peripheral, smaller arteries, though quite normal, were filled with granulation cells instead of with blood.

The source of the endarteritic infection is not discussed, but it is evident that the bacilli did not penetrate the arterial wall from without inward, but must have come with the blood.*

Ziegler observes that tuberculous meningitis is usually metastatic, but it may originate from adjacent structures. The tubercles may

* Of a somewhat different origin is the endarteritis described by Weintraud in the case of a man with pulmonary and laryngeal phthisis, who was taken with sudden nervous symptoms pointing to a local brain disease, but after eight days a fatal tuberculous meningitis supervened.

Microscopically, the intima of the arteries in the older tuberculous meningeal foci was concentrically thickened on account of the accumulation of spindle- and star-shaped cells lying in a homogeneous and fibrillary ground substance. There were occasional giant cells with traces of degeneration, but no tubercle bacilli, no tubercles, and no distinct caseation in the intima.

Weintraud declines to consider the disease of the intima as the primary process from which the meningeal infection followed, but he regards the endarteritis as secondary to a

develop anywhere in the pia, but the vessel walls are often involved to a marked degree, so that the structure is no longer recognisable on account of the caseation and the thrombosis.

Hamilton writes that the tubercles lie in the trabeculæ of fibrous tissue between the pia and the arachnoid, and that a small artery is generally found embedded in their substance or lying at their side.

The actual facts, gathered from this review of the literature concerning the arterial changes in tuberculous meningitis and their relation to the commencement of the process, may be summarized as follows:

The general opinion seems to be that the bacilli, carried by the blood, are localized in the arterial adventitia. The resulting process may invade the media and the intima, and the whole or a part of the wall may become caseous (Rindfleisch, Ziegler, Birch-Hirschfeld, Lancereaux, Guarnieri).

An endarteritis is also described whose relation to the other changes may vary. Some regard it as secondary (Guarnieri and Baumgarten), either due to direct extension or induced indirectly and somewhat in the same manner as chronic perivascular processes, tubercular or otherwise, call forth an obliterating endarteritis, a typical example of which Weintraud describes in a tuberculous pia.

Others regard the endarteritis as primary (Huguenin, Huettenbrenner, Cornil, Hirschberg), as due to the direct implantation of tubercle bacilli from the blood (Cornil, Huettenbrenner), and as the immediate source of the general infection of the pia (Hirschberg).

Concerning the histological technic used in this study, it is necessary to say simply this: That the paraffin-embedded pieces were cut into perfect series of sections from 5 to 10 μ in thickness, and that

latent meningitis in circumscribed cortical districts and as identical in its nature with the endarteritis obliterans of Friedländer, which may result from chronic inflammatory changes in the neighbourhood of vessels. Weintraud cites Rindfleisch, Friedländer, Baumgarten, Cornil and Guarnieri as having observed this or a similar form of endarteritis accompanying tuberculosis in the pia as well as elsewhere.

According to Guarnieri, Marchiafava, in 1876, directed attention to an angeiitis obliterans in chronic tuberculosis of the lungs, the brain, and the testicles.

the ordinary staining procedures were employed after affixing the sections to the slide with the water-albumin method.

In all cases the examination was directed especially to the arteries, particularly those in the Sylvian fissures.

The serial-section method presents obvious advantages that are indispensable in a study of this kind. Without perfect series of sections the relations of the arterial and the venous changes and of the extravascular and the intravascular changes to each other could not be clearly and perfectly understood in all the details.

Case I.—From Prof. Ganghofner's Pædiatric Clinic: Boy, five years old.

Clinical Diagnosis.—Basilar meningitis (miliary tuberculosis?); diffuse bronchitis; tuberculosis of the left elbow joint and of the cervical lymphatic glands. Section twenty-four hours after death (April 11, 1895).

Anatomical Diagnosis.—Basal and spinal tuberculous meningitis; chronic tuberculosis of the right lung, of the peribronchial lymph glands, of the cerebellum, of the right zygoma, of the left elbow joint, of the cervical and mesenteric lymphatic glands; bilateral fibrinous pneumonia; decubitus.

The body is 103 centimetres long, very emaciated. In the sacral region a 4-centimetre-large bedsore; about the left elbow joint are several fistulous openings that lead to rough bone. Over the right zygomatic arch opens a sinus that also leads to dead bone. The neck is thin, the thorax flat, and the abdomen retracted.

The skull is 51 centimetres in horizontal circumference, the inner surface being slightly roughened and the sutures somewhat loose. The dura is tense. The pia over the base contains a light turbid fluid, and around the vessels of the Sylvian fissures are numerous gray miliary granulations; here the exudate is more gelatiniform. The same changes are found about the arteries of the corpus callosum. On the upper surface of the cerebellum the meshes also contain miliary and submiliary nodules. The convolutions are flattened, and all the ventricles are dilated with turbid serum. The ependyma of the lateral ventricles is almost confluent. The brain substance is soft and moist. Along the anterior margin of the left cerebellar hemisphere is a caseous nodule, one cubic centimetre in size, which substitutes part of the cerebellar cortex, and is connected with the pachymeninx.

The nasal cavities are normal; the lining is covered with yellowish mucus. The thymus and thyreoid glands are of usual size. Many of the

cervical lymph glands are caseous. The right lung is universally adherent; the left is free. In the pleural adhesions over the right lung are numerous small caseous masses. The right lung is pale; in the lower part of the upper lobe is a cavity 2 centimetres in diameter, with caseous and fibrous walls; in the vicinity are caseous nodules. In both the lower lobes the parenchyma is hepatized without caseation. The heart is of normal size, the valves thin. The peribronchial glands are in part caseous.

The abdominal organs show no pathological changes, but the mesenteric glands are in part caseous.

The spinal pia is infiltrated with turbid serum, especially in the dorsal part, and contains many miliary nodules. The spinal cord is normal.

Bacteriological Examination.—Several cover-glass preparations of the meningeal exudate show no tubercle bacilli or other micro-organisms, and a glycerin-sugar-agar plate from the same exudate remained sterile after fifteen days in the incubator. Cover-glass preparations from the nose contain a large mixture of various bacteria, and among the numerous colonies on the plate cultures from the nasal muco-pus a staphylococcus staining with Gram's method seems the most prominent. In addition, cover glasses from the superior meatus on the left side contain a few tubercle bacilli.

Cover glasses from the pneumonic districts in the lungs show diplococci that take the Gram stain in rather small numbers.

Histological Examination.—The internal meninges present the usual changes, consisting of diffuse as well as more circumscribed cell infiltration, with comparatively many giant cells and occasional districts of caseation, and also much fibrinous exudate (Weigert's fibrin stain), all of which seems to be more marked at the base and in the beginning of the Sylvian fissures.

The caseation and the giant cells seem most pronounced in and around the adventitia of the smaller arteries, and quite frequently the infiltration and the caseation involve the entire media, either at only one point, or around the whole circumference of the vessel for a short distance longitudinally. In such instances there is usually more or less proliferation in the intima between the endothelium and the elastic lamina, but the latter usually remains intact. This invasion of the media with thickening of the intima is strongly

marked at the periphery of the older focus in the cerebellum. The accumulation of cells between the endothelial lining, which is well preserved, and the elastic layer usually consists of large epithelioid cells, with but occasional small lymphocytes and multinuclear leucocytes. Distinct tubercles and giant cells were not found upon the intima, and the changes in the external coats of the arteries usually seem older than those in the internal. Many of the smaller arteries are diffusely infiltrated and caseous, being, so to speak, directly transformed into tubercles. The main stem of the left Sylvian artery presents, at a point where it is free from adventitial changes, a nodular accumulation of lymphocytes and cells with multiform nuclei upon an intact intima.

The veins are frequently the seat of a marked infiltration which always seems to proceed from an artery or from changes in the neighbouring tissue; it is not unusual to find giant cells in the venous walls lying almost next to the blood stream. In one place a vein becomes the seat of two foci of circumscribed infiltration apparently by extension from a diffuse arteritis in two neighbouring arteries; the masses of granulation cells project into the lumen of the vein and become capped with thrombotic masses.

The internal meninges of the spinal cord show considerable exudation and infiltration which extends out into the nerve roots. In the arteries only the adventitia is usually involved, but the veins are often the seat of a diffuse proliferation with many giant cells; in some places the vein wall becomes very considerably thickened, and the lumen much narrowed. The walls of the minute arteries may be diffusely infiltrated. In addition to the giant cells—which are present in very large numbers and are remarkable for their size, the number of the so-called nuclei often reaching above one hundred, and the arrangement of the latter in the granular mass of the cell body forming at times a high wall at the periphery of the cell, at other times a heap in its centre or at one pole—three distinct kinds of cells are to be distinguished in the areas of proliferation: namely, small, round cells with comparatively large nuclei which stain deeply, and with a very small cell body (small lymphocytes);

and large cells of circular form, with relatively small nuclei and a finely granular, almost homogeneous cytoplasm (epithelioid cells—lymphatic cells, Metschnikoff); and, finally, cells with polymorphous or fragmented nuclei found wherever caseation is going on.

Tubercle Bacilli.—These are easily demonstrable in large numbers in the old focus in the cerebellum and in the adventitial proliferation in both the brain and the cord. In sections from the cortex of the central convolutions bacilli are also present in small numbers in the interior and in the wall of small arteries with inflammatory changes. Bacilli were also found in the media and in the interior of small arteries with changed walls in the spinal pia.

Case II.—From Prof. Ganghofner's Pædiatric Clinic: Girl, seven and a half years old.

Clinical Diagnosis.—Tuberculous meningitis. Section eighteen hours after death (April 20, 1895).

Anatomical Diagnosis.—Tuberculous meningitis; chronic tuberculosis of the peribronchial lymphatic glands, of the brain, the thyreoid gland, the lungs, the liver, the spleen, the kidneys, the stomach, and the intestines.

The body is 103 centimetres long, of feeble build, and greatly emaciated. The rigor mortis is slight. The hair is brown, the pupils equal, the neck thin, the thorax flat, and the abdomen retracted.

The skull is 48 centimetres in horizontal circumference; the sutures are somewhat loose. The dura is tense; the dural sinuses contain fluid and clotted blood. The pial vessels are full of blood; at the base, especially about the optic chiasm, is much turbid, serous fluid; along the vessels of the pia, both at the base and over the surface, are numerous miliary tubercles lying in fibrinous exudate. The brain substance is moist and soft, especially near the ventricles, which are much dilated with turbid fluid. Here and there the cortex of both hemispheres of the brain contain caseous nodules about the size of a pea, which are connected with the pia. The nasal mucous membrane is covered with thick yellow muco-pus.

The thymus gland is of usual size. The thyreoid is rather large, and contains numerous areas of caseous infiltration.

The laryngeal and tracheal mucous membrane is covered with yellow mucus. Both lungs contain numerous yellowish-white nodules, some as large as a pea. In the peribronchial lymph glands are caseous and calcareous masses. The pericardium contains a tablespoonful of serum. The heart is of usual size, and its valves are thin. The liver contains numerous caseous nodules up to 0·5 centimetre in diameter, with softened, bile-

stained centres. The spleen is somewhat large, and contains caseous masses. The kidneys contain a few scattered miliary tubercles. The genital organs are normal. In the stomach are found occasional caseous nodules as large as a split pea, some of which present ulceration on the mucous surface. In the inferior ileum and in the cæcum are caseous nodules and also small ulcers. The pancreas and the adrenals are normal.

Bacteriological Examination.—The cover glasses from the meningeal exudate contain a few staphylococci and occasional tubercle bacilli.

Plate cultures show, after thirty-six hours, three pinhead-sized, grayish, round colonies of staphylococci that stain with Gram's method, present a thick, grayish-white growth on slanting agar, liquefy gelatin, and make bouillon turbid.

The cover glasses from the nose contain a mixture of various bacteria, staphylococci being present in large numbers. Tubercle bacilli are not found.

Plate cultures from the upper as well as from the lower meatus contain mostly staphylococci identical with that from the meningeal exudate.

Histological Examination.—In this case the changes are very intense, as marked by the dense cell infiltration and by the extensive degeneration. Giant cells are frequently present, especially in the peri-arterial and para-arterial foci and in the walls of veins. The inflammation in places involves the superficial layers of the brain cortex and extends also inward in the perivascular tissue of the intracerebral vessels. In many places the peri-arterial infiltration and caseation is accompanied by a hyaline degeneration of the media, whose inner layers, especially, then present a homogeneous, structureless appearance, the intima usually showing some subendothelial proliferation. The wall of the left Sylvian artery presents at one point a circumscribed infiltration, most marked in the adventitia and the outer layers of the media, but reaching the intima. Farther out in the Sylvian fissure there is quite a marked subendothelial proliferation in the large arteries with but slight if any changes in the media or the adventitia; the elastic layer remains intact, and there

are no tubercles or giant cells upon the intima. The arteries occasionally contain thrombi, in which may be accumulations consisting essentially of multinuclear leucocytes.

The smaller arteries are often diffusely involved and changed into small tubercles. Circumscribed and diffuse infiltration into the walls of large as well as small veins, with frequent giant cells, is everywhere seen (Plate VIII, Fig. 6). From the serial sections it is easy to trace the affection of the veins to invasion from the neighbourhood.

Tubercle bacilli are present in very large masses in the peri-arterial tuberculous infiltration; they are also found in smaller numbers in the walls of the smaller arteries.

Case III.—From Prof. Ganghofner's Pædiatric Clinic: Girl, four and a half years old.

Clinical Diagnosis.—Tuberculous meningitis. Section fourteen hours after death (April 21, 1895).

Anatomical Diagnosis.—Basilar and spinal tuberculous meningitis; chronic tuberculosis of the peribronchial lymph glands, of the brain, and of the fourth and fifth lumbar vertebræ; miliary tuberculosis of the lungs, the liver, and the spleen; lobar pneumonia; multiple cartilaginous exostoses.

The body is 92 centimetres long, of feeble build, and much emaciated. The rigor mortis is slight. The hair is light, the neck thin, the thorax long, and the abdomen retracted. There are a large number of cartilaginous exostoses in the vicinity of the lines of ossification in nearly all parts of the body.

The skull measures 47·5 centimetres in its greatest horizontal circumference. It is thin, the inner surface is a little rough, and the frontal suture is persistent. The dura is somewhat tense. The pia is hyperæmic, and its meshes contain much thick exudate, especially at the base and in the beginning of the Sylvian fissures. Along the vessels, as well as elsewhere, are numerous miliary and submiliary nodules. The ventricles are dilated and filled with turbid serum. The brain substance is swollen and moist, the convolutions being greatly flattened. In the left cuneus and in the centre of the left first temporal convolution are caseous nodules, each the size of a pea. In both the cerebellar hemispheres are found several nodules up to 0·5 centimetre in diameter. In the pons above the left locus cæruleus is also a small nodule; in the same section there is a caseous mass 0·5 centimetre large in the ventral portion of the right half of the pons.

The mucous membrane of the nose, the pharynx, and the larynx is pale. The thyreoid and thymus glands are of usual size. The lungs are hyperæmic and œdematous, and contain numerous miliary tubercles. The left lower lobe is hepatized. In the bronchi is considerable muco-pus. The peribronchial lymph glands are caseous. The heart is of usual size, the valves thin. The liver and the spleen contain miliary tubercles. The stomach, the intestines, and the genito-urinary organs are normal. In the left psoas muscle is a large abscess which occupies the entire extent of the muscle; in the fourth and fifth lumbar vertebræ are found areas of caries. The spinal pia is hyperæmic, infiltrated with serum, and here and there are miliary tubercles.

Bacteriological Examination.—Cover glasses from the meningeal exudate contain isolated tubercle bacilli. Plate cultures from the meningeal fluid remain sterile.

Tubercle bacilli are not found in the mucus from the nose.

Histological Examination.—In the Sylvian fissures the changes are very intense, the vessels being embedded in the diffuse infiltration and the dense fibrinous exudate. The media of the larger arteries is usually diffusely infiltrated, the lumen frequently thrombotic, and along the tunica elastica is a layer of various kinds of cells with detritus and nuclear fragments, the endothelium being absent. The perivascular infiltration is in process of degeneration, and this extends into the media and at times through the entire wall.

In other places there is a well-marked peri-arteritis and mesarteritis with more or less hyaline degeneration in the media and with accumulations of leucocytes upon an intact endothelium.

The veins show, as usual, extensive tubercular infiltration into the walls; in one place a polypoid mass of granulation cells projects from the wall into the lumen; in another place the sections of a large vein present a circumscribed infiltration into the wall, which appears to be undergoing disintegration, so that the internal surface presents an appearance that might be referred to as a tuberculous ulcer. Giant cells are frequent in the walls of veins, rare in arteries.

Tubercle bacilli are found in large numbers in the more recent areas of proliferation. The character of the tissue is no sure indication as to the presence of tubercle bacilli, which are easily demon-

strable in large numbers in one place and difficult to find or absent in another, the histological appearances being the same in both.

In a small artery in the Sylvian fissure, which presents a circumscribed proliferation at one point, tubercle bacilli are found in the lumen of the vessel next to the intima. Tubercle bacilli are also present in the wall, and free in the lumen, of a small infiltrated vein of the pia over the central convolutions of the left side.

Case IV.—From Prof. Ganghofner's Pædiatric Clinic: Girl, ten months old.

Clinical Diagnosis.—Tuberculous meningitis. Section eighteen hours after death (April 24, 1895).

Anatomical Diagnosis.—Basilar and spinal tuberculous meningitis; chronic tuberculosis of the peribronchial glands, the pericardium, the liver, the leptomeninx, the brain, and the cranium.

The body is 70 centimetres long, well nourished, with livid discolorations posteriorly. Rigor mortis present. The hair is blond, the pupils equal, the neck short, the thorax oval, and the abdomen slightly distended. The skull measures 42 centimetres in horizontal circumference; it is of usual thickness; the sutures are loose; the large fontanelle is 4 centimetres large. In the right inferior quadrant of the left temporal bone is an area, 2 centimetres square, in which the bone is infiltrated with caseous material which invades the periosteum and the pachymeninx. In the sinuses of the dura is fluid blood. The pia does not contain much blood; at the beginning of the Sylvian arteries and the arteries of the corpus callosum are several pea-sized nodules in the pia which extend into the cortex; in addition there is much serous and gelatiniform exudate, and also numerous miliary nodules along the vessels of the Sylvian fissures as well as elsewhere. The brain substance is pale, quite soft, especially around the dilated ventricles, where it is almost confluent. The fourth ventricle is also somewhat dilated. The lining of the nose is covered with thick mucus.

The trachea contains fluid from the stomach. The thymus and the thyreoid are of normal size. The lungs are not adherent; slightly œdematous. The epicardium is thickened, and contains single and conglomerate caseous nodules as large as grains of wheat; at the upper part of the pericardium are adhesions between its two layers. The heart is of usual size, the valves thin, the muscle pale. The peribronchial lymph glands are enlarged and in part caseous.

The liver contains caseous nodules, as large as a pea, with softened centres. The spleen is of usual size. The kidneys are pale, the bladder

and genitalia normal. The mucous membrane of the stomach is softened in the pyloric region. The intestines are normal. The mesenteric glands are enlarged but not caseous. The spinal pia contains numerous small tubercles.

Bacteriological Examination.—Cover-glass preparations from the meningeal exudate do not contain tubercle bacilli or other bacteria, and plates with the same material remain sterile at the end of three weeks in the thermostat. No tubercle bacilli were found in the nose.

Histological Examination.—The larger arteries at the base, such as the Sylvian and the arteries of the corpus callosum, show the most intense involvement of the entire wall in infiltration and caseation, so that the structure of the arteries is no longer recognizable. The elastic layer seems to remain distinct the longest. This extensive affection of the wall is accompanied with similar violent changes in the neighbourhood. Upon the internal surface of the arteries occur frequent nodular accumulations of cells and detritus, covered with fibrin, but the degeneration is so advanced that one can not distinguish between possible miliary tubercles and parietal leucocytic masses.

At a point where the tuberculous process is not so intense the left Sylvian artery proper presents a small, sharply circumscribed perforation filled with granulation tissue, and the changes in the intima are certainly as old at this point as those in the adventitia. The walls of the adjacent smaller vessels usually show a total hyaline degeneration with thrombosis.

In the Sylvian fissures the changes are also very marked; the infiltration into the adventitia and the media is usually accompanied with endarteritis, the media remaining at times nearly normal, at times becoming homogeneous and hyaline. The subendothelial proliferation usually appears most marked at the points of bifurcation of the arteries.

In some places the entire segment of an artery may be totally necrotic. The veins present infiltration and caseation in the same high degree as the arteries. The meningeal process frequently involves the subjacent cortex.

The old foci in the pia and the subjacent cortex are marked by the presence of a very large number of large giant cells and by severe vascular changes.

In many places the spinal leptomeninx shows a sparse infiltration, especially of epithelioid cells, into the lymph spaces, the walls of the veins, and the arterial adventitia. The number of large giant cells is considerable; they sometimes lie in the venous wall next to the lumen. The giant cells here present an appearance as though they were formed by the arrangement of nuclei in a homogeneous or finely granular mass that sends prolongations in all directions out among the adjacent cells.

Tubercle bacilli are present in large masses in the wall of the Sylvian artery and in the arterial adventitia elsewhere.

Case V.—From Prof. Ganghofner's Pædiatric Clinic: Girl, eight years old.

Clinical Diagnosis.—Broncho-pneumonia; miliary tuberculosis. Section nine hours after death (April 26, 1895).

Anatomical Diagnosis.—Chronic tuberculosis of the peribronchial lymphatic glands of the right pleura and lung; universal subacute tuberculosis; tuberculous meningitis; subcutaneous emphysema.

The body is 166 centimetres long, of slender build, and much emaciated. The hair is brown; the pupils equal. In the left supraclavicular fossa is subcutaneous emphysema that extends into the left axillary space; there is emphysema of less degree in the left supraclavicular fossa. The abdomen is somewhat retracted.

The skull measures 47 centimetres in its horizontal circumference. The dura is tense; its sinuses contain fluid and clotted blood. The pia over the forebrain is hyperæmic and œdematous; at the base, and especially along the arteries of the corpus callosum, are groups of millet-seed-sized and larger caseous nodules; here and there upon the convexities are occasional small yellowish masses around the vessels. The convolutions are flattened, the ventricles dilated with lightly turbid serum, the ependyma softened. The thyreoid contains many miliary nodules. The thymus is small.

The right lung is totally adherent to the chest wall, and in the adhesions are many miliary tubercles and caseous nodules; in the lung parenchyma are similar masses. The left lung is free, the cut surface displaying miliary nodules. The pericardium is adherent to the right visceral pleura, and contains a tablespoonful of clear serum. The heart is of normal size, the valves thin.

The peribronchial glands are enlarged and in part caseous. In the liver, the spleen, and the kidneys are many miliary tubercles. The stomach, the intestines, and the genital organs are normal.

Bacteriological and Histological Examination.—Cover-glass preparations from the meningeal exudate contain only tubercle bacilli, which are present in large numbers. The bacilli occur most frequently in the interior of large, roundish cells, at times singly, at times in groups of four, six, ten, or more. Occasionally a bacillus is found lying directly across or upon the nucleus of the cell, but usually the bacilli occupy the cell body.

These large cells are very numerous. They usually have but one nucleus, but cells with two or more nuclear bodies occur. Many such cells containing bacilli are necrotic; frequently the nucleus is split up into irregular fragments or replaced by round masses of varying size—karyolysis; detritus masses of the shape of disintegrated cells are seen, and among the remnants may lie tubercle bacilli.

Free tubercle bacilli, singly, but oftener in heaps or groups, are also found.

In addition to apparently well-preserved as well as disintegrating cells, the meningeal fluid contains chromatin masses of varying size and shape, and also leucocytes with polymorphous nuclei.

Glycerin-sugar-agar plates made with the meningeal fluid remain sterile. The nose could not be examined.

Serial sections through the smaller caseous masses in the pia over the convexity present the following appearances: Through the degenerated centre run one or two arteries whose adventitia and media are infiltrated and caseous, sometimes homogeneous and hyaline, the intima being the seat of a slight subendothelial proliferation, the lumen frequently thrombotic. Sometimes small hyaline masses occur in the intima. The arteries are surrounded on all sides by caseous material in which lie scattered fragments of nuclei and polynuclear leucocytes. The walls of the adjacent veins are invariably infiltrated; often the lumen is partially or wholly occluded by thrombotic masses whose interior present foci of leucocytic infiltration.

The process also involves the upper layers of the subjacent cortex. Toward the periphery of such a mass or tubercle the adventitia is more loosely infiltrated with large and small cells, the media usually quite normal, but the intima usually showing some subendothelial proliferation. Giant cells were not found in these areas; Weigert's fibrin stain demonstrates considerable fibrin in their peripheral parts. The neighbouring lymph spaces are loosely filled with epithelioid cells, much like those found in the meningeal exudate, and contain free and intracellular tubercle bacilli in large numbers.

Circumscribed, minute, tuberculous foci are also found in the interior of the brain without any demonstrable connection with the larger vessels.

The arteries of the corpus callosum and their branches present the following appearances: The lumen is more or less occluded with thrombotic masses that often contain large accumulations of leucocytes. The endothelial lining is either absent or the cells lie loose and disarranged. Frequently the internal wall presents nodular masses of cells, which penetrate the elastic layer and invade the media in a semicircular manner, the whole presenting a tubercle one half of which extends into the lumen, the other half into the wall of the artery; the centre of such masses is usually caseous, but in one or two instances distinct giant cells are present.

At one point the flattened artery is totally occluded by a large mass of cells and detritus in which lie several quite well-preserved, multinucleated giant cells; at the side of the mass that borders upon the main lumen of the artery lie several endothelial cells.

Around this intravascular process the arterial wall presents diffuse inflammatory and degenerative changes which at times render the structure indistinct. Corresponding to such foci in the arteries are diffuse and extensive changes in the surrounding tissues which invade the cortex, but the only giant cells found are those in the intravascular tubercles. The walls of the smaller vessels that are embedded in such masses are totally caseous or hyaline.

From older foci the process seems to extend along in the ad-

ventitia of the arteries, and at times a circumscribed invasion of the media may occur.

The sections removed from the series of these large arteries for the purpose of demonstrating the tubercle bacilli did not happen to include any extensive intravascular changes. Bacilli are present in enormous numbers in the wall of as well as outside the arteries. Frequently dense heaps or masses of bacilli are found that are recognisable as violet spots (Ehrlich's method) with a low power.

Case VI.—From the Medical Clinic of Prof. Pribram: Man, aged forty-one.

Clinical Diagnosis.—Tuberculous meningitis; sero-fibrinous pleuritis; pulmonary emphysema; apical infiltration in the lungs; arterio-sclerosis; chronic alcoholism. Section twenty-four hours after death (May 17, 1895).

Anatomical Diagnosis.—Chronic tuberculosis of the lung apices; right tuberculous pleuritis; chronic tuberculosis of peribronchial lymph glands; universal miliary tuberculosis; basilar tuberculous meningitis; fibrous orchitis.

The body is 170 centimetres long, the bones are light, the muscles poorly developed, the panniculus adiposus slight. Diffuse posterior lividities. The hair is thick, brown; the pupils equal; the neck of medium length; the thorax rather flat, the right half larger than the left. The abdomen is prominent. The skull is thick, 56 centimetres in circumference. The dura is tense, the sinuses containing fluid blood. The vessels of the pia are filled with blood, the meshes are œdematous and infiltrated with clear serous exudate, and along and upon the vessels are numerous minute grayish-white nodules. The brain substance is moist, the ventricles somewhat distended with clear fluid.

The mucous membrane of the organs of the neck is normal but pale. The thyreoid is apparently normal. The left lung is adherent; in its apex is an old scar. The right pleural cavity contains more than one litre of sero-fibrinous fluid, and both pleural layers are covered with fibrinous exudate and tubercles. The right lung is pale and compressed; in the apex is an old calcified cicatrix. In both lungs the bronchi contain mucopus, the parenchyma being the seat of miliary tubercles. The pericardium contains a tablespoonful of fluid. The heart muscle is flabby and easily torn; the valves are thin; the aorta is smooth.

The œsophagus is normal. The peribronchial glands are large and caseous. The liver, the spleen, the kidneys, the pancreas, and the adrenals contain numerous miliary nodules. The testicles are hard, both layers of the

tunica vaginalis are adherent, and the parenchyma, especially in the right testis, is replaced by hard, white, fibrous tissue.

The stomach is dilated and contains many hæmorrhagic erosions; the ileum and the colon are normal.

Bacteriological Examination.—Cover-glass preparations from the meningeal exudate contain tubercle bacilli in small numbers. Other bacteria are not present.

Plate cultures from the exudate remain sterile after three weeks in the thermostat.

Serum and glycerin-agar tubes inoculated with a drop or two of the meningeal effusion develop slowly-growing cultures of tubercle bacilli. The nose could not be examined.

Histological Examination.—The tissues from this case presented astonishingly slight post-mortem changes, and proved an excellent material for the study of the vascular changes which involve the internal coats of the arteries to a remarkable degree.

More or less acute endarteritis is present in almost all the arteries, large as well as small. In the large arteries, such as the main stem of the Sylvian, the process is usually limited to one part or segment of the internal circumference of the vessel. In the medium-sized arteries the proliferation oftenest extends completely around the vessel, and the smaller arteries are frequently more or less completely occluded by the loosely arranged accumulation of cells in their interior. It is in the medium-sized arteries that the specific characters of the process are best studied.

The circumstance that the endothelium is unusually well preserved renders it easy to recognise that the proliferation is invariably subendothelial. It is only under the special circumstances to be mentioned that the endothelial lining is absent. When the cell proliferation completely fills the lumen of the vessel, then the endothelial lining will be found in the central part, at times disarranged and rolled together, at other times undisturbed.

The endarteritic proliferation is made up of cell layers of varying thickness. Large round or oval cells with comparatively rather small nuclei are the most numerous and predominating elements.

Smaller cells with large, deeply stained nuclei and cells with polymorphous or fragmented nuclei are also occasionally present. In some places one meets with cells containing three or four nuclei, as well as with multinucleated giant cells, and this outside of the more typical tubercles to be described (Plate VIII, Figs. 1 and 2).

Generally speaking, there is no very distinct intercellular substance; in some places a fibrinous and granular exudate is present in small quantity, especially in the deeper layers of the more advanced proliferation.

A well-preserved endothelium composed of a single row of typical endothelial cells limits the inflammatory proliferation on one side and the elastic layer (or the media) limits it on the other. There are absolutely no signs in the endothelium or disturbances of its arrangement which would indicate any active changes on its part. The nuclei of the endothelial cells are not swollen and present no appearances indicative of karyokinesis.

At times the elastic layer is invaded by cells, and an infiltration of variable degree takes place in the media and in the adventitia, but one can also examine large series of sections without finding any changes in the elastic tunic, the media, or the adventitia. Then again an unchanged media may intervene between the endarteritic and the familiar peri-arteritic infiltration. In the smaller arteries the elastic layer may be completely absent. Usually the arteries are not thrombotic.

At frequent intervals the more uniform and diffuse proliferation that has been described is intensified, as it were, by the formation of more or less distinctly nodular cell masses in which may be found giant cells and caseation. It is over such caseous intimal tubercles that the endothelium may be absent (Plate VIII, Fig. 4). The cells in such tubercles apply themselves firmly to the arterial wall and appear closely packed together. The multinucleated giant cells usually occur in the centre of the little nodules; round about are epithelioid and small cells, and, as caseation becomes marked, more polynuclear leucocytes appear.

Close study of the beginning of this subendothelial, intimal pro-

liferation shows that at first one or two cells appear in the intima here and there. A little later minute nodular accumulations of epithelioid cells have formed which lift the endothelium away from the intima, so that the endothelial lining presents a series of longitudinal folds corresponding to the subendothelial cell masses. The cells in such places present many evidences of proliferation in the shape of cells in the act of division, cells with two nuclei, and cells with more or less indistinct but nevertheless unmistakable karyokinetic nuclear figures.

Any other disarrangement of the endothelium in these places than the mechanical one described is usually not observed, and the elastic layer and the media are unchanged.

The elastic lamina underneath the tubercles may be quite intact, more or less infiltrated with cells, exudate and detritus, or entirely and sharply perforated as if a hole had been punched in it. Sometimes exudate may lift the ends of the perforated layer away from the media (Plate VIII, Fig. 4).

Corresponding to the tubercles, the media and the adventitia may be quite normal, or they may show infiltration and caseation of all grades of intensity.

Cell masses with as well as without giant cells or caseation may occur in the intima of arteries where there are no other changes, either intravascular or extravascular, but this is not common.

The walls of veins, large and small, show diffuse and circumscribed infiltration, but without giant cells.

The extravascular changes proper in this case consist of moderately extensive diffuse, as well as more circumscribed, infiltration, with but little caseation and no giant cells. These changes are usually better marked in the pia itself than in the arachnoidal layer.

There were no foci found in the interior of the brain substance.

Tubercle bacilli are found in very small numbers in the intimal tubercles; but in many sections through such masses, including the giant cell, the search for bacilli was entirely in vain. Many small, round, and rod-shaped intracellular granules were found that retained

the stain like the bacilli. In one place bacilli were found in the elastic layer, but occasional bacilli were found in the diffuse intimal proliferation.

Bacilli were also found in the infiltrated walls of veins, and, in larger numbers, in the peri-arterial infiltration.

Case VII.—From the Psychiatric Division of Dr. Zastera: Woman, aged twenty-six.

Clinical Diagnosis.—Melancholia, intestinal and pulmonary tuberculosis. Section thirty-six hours after death (May 29, 1895).

Anatomical Diagnosis.—Tuberculous cerebro-spinal meningitis; chronic tuberculosis of the peribronchial lymph glands, of the lungs, the liver, the spleen, and the kidneys. Tuberculous pleuritis.

The body is 167 centimetres long and quite emaciated. Rigor mortis is present. The hair is light; the pupils equal and wide; the visible mucous membranes pale; the neck is thin; the thorax long and flat. The dura is tense, and the sinuses contain liquid and clotted blood. The pial vessels are full of blood, and the meshes are distended with turbid serum, especially at the base about the beginning of the Sylvian fissures. Along the vessels, principally those in the Sylvian fissures, are many small gray nodules; on close examination some nodules appear to be situated in the walls of the vessels. The brain substance is moist and soft; the convolutions are flattened; the ventricles are filled to dilatation with turbid serum, the ependyma being softened.

The mucous membrane of the nose and of the pharynx is covered with yellow, viscid mucus. The thyreoid is of normal size. The pericardium is empty, the heart small and brown. The right pleura is covered with fibrinous exudate, in which are miliary nodules, and in the cavity is about one litre of clear serum. In both lung apices are circumscribed tubercular foci. The peribronchial lymph glands contain caseous masses. In the liver are caseous nodules which have a softened, bile-stained centre. The capsule of the spleen contains caseous masses. The kidneys are small, smooth, and pale brown; in the interior are scattered small yellowish nodules. The genito-urinary mucous membranes are normal. The stomach, the intestines, the pancreas, and the adrenals are normal. In the spinal pia are occasional miliary granulations and some infiltration with turbid serum.

Bacteriological Examination.—Cover-glass preparations from the meningeal exudate contain tubercle bacilli often in heaps of four to six, and usually free. No other bacteria are present.

Plate cultures from the exudate remain sterile at the end of some

days in the thermostat, but blood-serum tubes present pure growths of tubercle bacilli.

The injection of 0·3 cubic centimetre of ventricular fluid through a small trephine opening into the subarachnoid space of a rabbit produced in two weeks a solitary tubercle 0·8 centimetre large in the subjacent brain with meningeal adhesions, accompanied with great emaciation, but without fever; there was no meningeal exudate and no admixture of other bacteria.

Cover-glass preparations from the nasal muco-pus contain staphylococci, streptococci, diplococci, and occasional tubercle bacilli.

Histological Examination.—The arterial changes in this case present many points of interest that may be summarized as follows: The serial sections through pieces of the Sylvian arteries, including the tubercles that seemed to sit in the arterial wall, show a distinctly circumscribed and focal infiltration of all the layers of the wall, with some caseation and considerable thickening of the intima underneath the endothelium (at times this is absent), often accompanied with a sharp, punched-out perforation of the elastic layer and a more or less precisely circumscribed infiltration in the media, the adventitial changes being at times marked, at times very insignificant, or even entirely absent (Plate VIII, Fig. 5). Now it happens that in such foci as these there may be a distinct outward bulging of the vessel wall corresponding to the infiltration in the media, the ends of the elastic lamina being turned outward on each side of the perforation, the whole representing to some extent a minute aneurism in which the arterial wall is more or less completely substituted by tuberculous granulation tissue. Many such peculiar foci are found not only in the branches in the Sylvian fissures, but also in the arteries of the convexity. In some places only the innermost portions of the granulation tissue (the part between the ends of the elastic lamina and in the intima) is caseous; giant cells are not present. Rupture with hæmorrhage of any such perforating focus was not found.

Isolated intimal masses of cells without changes in the media or in the adventitia are also found, and at times they show distinct caseation; some doubtful giant cells are also seen.

Furthermore, the vessels present a marked endarteritis both with as well as without changes in the other walls, leading often to a more or less complete occlusion of the lumen. Occasionally the subendothelial intimal proliferation breaks through the elastic layer and spreads out between this and the media.

A smaller series of sections through the cerebellar cortex includes a focus of endarteritis in a large vessel. As is usually the case, it is distinctly subendothelial, and the cells are mostly large and round with some scattered lymphocytes. A distinct intercellular substance is not observable. At the point where most marked it produces occlusion of one part of the lumen; here the cells of the endothelial layer meet each other in the centre of the vessel. There is a very slight infiltration with some hyaline degeneration in the inner layers of the surrounding media with barely perceptible peri-arterial changes. There are occasional cells with two to four nuclei, but no distinct giant cells.

In places where the changes are diffuse the smaller arteries are totally infiltrated, caseous, or hyaline. The walls of the veins are usually extensively infiltrated, and at times the lumen contains thrombotic masses. In the spinal pia there is some infiltration into the arterial adventitia and into the walls of veins; there are no special changes in the intima of the arteries.

In all the sections from this case the only indications of giant cells observed after vigilant search were certain irregular masses with many nuclei in the intravascular foci. Tubercle bacilli were found in small numbers in the intimal proliferations, and somewhat more numerously in the extravascular granulation tissue.

Case VIII.—From the Medical Clinic of Prof. Pribram: Woman, aged twenty-four.

Clinical Diagnosis.—Tuberculous meningitis; lumbar spondylitis. Section twelve hours post mortem (June 12, 1895).

Anatomical Diagnosis.—Obsolete tuberculosis of the peribronchial lymph glands; chronic tuberculosis of the second and third lumbar vertebræ; psoas abscess; universal subacute tuberculosis; tuberculous meningitis.

The body is 160 centimetres long, of feeble build and poor nourishment. Pale lividities posteriorly. The neck is narrow and long. The chest is flat and narrow. The abdomen is retracted.

The skull is thin, 50 centimetres in horizontal circumference. The dura is tense and in the sinuses is fluid blood. In the meshes of the pia, especially about the base, but also over the upper surface of the brain, is a turbid, sero-fibrinous exudate. Along the Sylvian arteries on both sides are innumerable minute, grayish, semi-transparent nodules, which appear to sit partly upon, partly in the walls of the vessels. Similar nodules are also found along the vessels of the convexity, the choroid plexuses, and in the floors of the lateral ventricles, but in much smaller numbers. Upon the left half of the brain are a few a little larger and yellowish masses in the pia of irregular form which extend into the brain substance.

In the thyreoid gland are numerous miliary nodules. Both lung apices are slightly adherent. Throughout the pulmonary parenchyma of both sides are distributed numerous minute yellowish nodules of equal size. The heart is small, its muscle dark red, the valves thin.

The peribronchial lymph glands contain calcareous and fibrous masses. The capsule and the parenchyma of the liver contain numerous nodules which are also found in the spleen and in the kidneys. The genital organs, the stomach, the intestines, the pancreas, and the adrenals are without any special changes. In the bodies of the second and third lumbar vertebræ are caseous masses, and in the right psoas major muscle is a small abscess. The spinal cord and its membranes are without any changes.

Bacteriological Examination.—Cover-glass preparations from the meningeal exudate contain now and then single tubercle bacilli and no other micro-organisms.

Glycerin-sugar-agar plates inoculated with the meningeal exudate remain sterile after some days in the thermostat. Blood-serum tubes inoculated with the same exudate showed pure cultures of tubercle bacilli.

Histological Examination.—In some places, especially at the beginning of the Sylvian fissures as well as in the apparently somewhat older masses upon the surface of the left hemisphere, there is much fibrinous exudate and diffuse cell proliferation with caseation. Here the smaller arteries are diffusely and wholly caseous, at times hyaline. The so-called hyaline degeneration may involve the whole or part of the wall, usually the media, in the form of a homogeneous ring which stains a bluish-violet tinge with hæmatoxylin (Delafield's) and bright red with Van Gieson's stain. Hyaline masses of irregular shape are also found in the other coats of the vessels and in the

extravascular infiltration. The larger arteries frequently show a uniform and intense involvement of the adventitia and of the outer layers of the media; in the inner layers of the media the infiltration is at times diffuse, the cells being rather sparsely scattered about; at other times the infiltration is more focal and denser, with occasional cell accumulations between the elastic layer and the media. There is usually, but not constantly, a diffuse and pronounced subendothelial proliferation of large and small round cells, lying sometimes in a granular, sometimes in a slightly fibrillated matrix; frequently there are more nodular cell masses, in the centre of which is some degeneration, and also probable necrotic giant cells. Here and there the elastic layer is split up by infiltrating cells. Lymphoid tubercles with giant cells are also found upon the intima of the larger arteries, with but moderate general endarteritis, the media and the adventitia being the seat of a not very marked infiltration.

Further out in the Sylvian fissures the arteries present circumscribed nodular cell infiltration with some degeneration which involves all the coats in a fairly equal degree. More or less endarteritis is present here also. In the smaller arteries are observed focal intravascular and extravascular changes usually connected, as it were, by a mild infiltration through the media, but frequently entirely independent of each other.

Serial sections through minute masses in the pia over the brain surface show the cell mass to be connected with a small artery, one segment of its wall being completely involved in the tubercle, which seems to arrange itself in a concentric manner about this part of the vessel. Some slight and diffuse endarteritis is also present. In the centre of the tubercle the degeneration seems about as far advanced in the inner as in the outer parts of the wall involved, but at the periphery the degeneration is most marked in the peri-arterial infiltration.

The beginning of such foci as the last is probably illustrated in the small, circumscribed, subendothelial, intimal proliferations with giant cells accompanied with only slight infiltration into the media and the adventitia, and with the formation of epithelioid cells in the

peri-arterial lymph spaces, the intimal changes being by far the most pronounced which are occasionally found in the pial arteries of the convexity.

The veins are usually the seat of a diffuse infiltration; at times an apparent isolated endophlebitis is observed, but sooner or later such veins present points where the whole wall is involved.

Tubercle bacilli are present in some numbers in the endovascular foci, but most numerously in the peri-arterial infiltration.

Case IX.—From the Psychiatric Clinic of Prof. A. Pick: Man, forty-six years old.

Clinical Diagnosis.—Delirium; meningitis cerebro-spinalis. Section ten hours after death (May 24, 1895).

Anatomical Diagnosis.—Chronic tuberculosis of the lung apices and of the peribronchial lymphatic glands; tuberculous meningitis; universal miliary tuberculosis.

Strongly built man with well-developed musculature, the body being 174 centimetres long. Rigor mortis strong. The pupils are equal, the thorax oval, and the abdomen retracted.

The skull is 56 centimetres in horizontal circumference, rather thick, and its internal surface somewhat roughened. The dural sinuses contain a moderate quantity of fluid blood. The internal meninges are rather pale; about the base the meshes contain turbid serum. Along the Sylvian arteries are numerous grayish nodules the size of millet seeds. Similar nodules are also present upon the upper surface of the cerebellum. The brain substance is moist. In the neighbourhood of the dilated ventricles, which are filled with turbid fluid, the substance is softened. The mucous membrane of the organs of the neck is pale. The thyreoid is rather small. In the apices of both lungs are older caseous and calcareous masses, surrounded by dense cicatricial tissue. The parenchyma contains everywhere numerous miliary, grayish-yellow nodules. The peribronchial glands are partly caseous. The pericardium contains a small quantity of serum. The heart is of normal size, pale, the valves thin. The liver and the spleen are the seat of innumerable miliary tubercles. The kidneys are of usual size, and the cortex contains numerous miliary nodules. The genital organs, the stomach and the intestines, the pancreas, and the adrenals show no pathological changes.

Bacteriological Examination.—Cover glasses from the meningeal effusion contain a very few tubercle bacilli, but no other bacteria.

Glycerin-sugar-agar plates from the same exudate remain sterile. The nose could not be examined.

Histological Examination.—There is a moderately extensive extravascular infiltration, with some caseation, but no giant cells. The adventitia of the smaller arteries is diffusely and densely infiltrated with epithelioid cells and small round cells. At times the media remains normal, at other times it is infiltrated, but in this case there is no hyaline degeneration of the media observable. In these arteries there is also a diffuse subendothelial proliferation that sometimes nearly fills the lumen of the vessels. Small arteries diffusely involved and changed into miliary tubercles are also present.

In the medium-sized arteries there may be considerable endarteritis, occasionally leading to a nearly complete occlusion of the lumen, without changes in the other coats (Plate VIII, Fig. 3), except occasional foci in the periphery of the adventitia; then again the endarteritic changes may be associated with more extensive and dense adventitial proliferation.

The large arteries present occasional distinct tubercles located underneath the endothelium, which may be absent at but one point corresponding to which there is no thrombosis. Such a tubercle is usually accompanied with some endarteritis which extends half way or so around the vessel. At various points the cells of the tubercle may pass through the elastic lamina and infiltrate the media. In the adventitia there may be some proliferation corresponding to the tubercle, but only of slight degree. In places the subendothelial intimal proliferation commences with the formation of cells upon the elastic lamina; in some of these cells the nucleus is large and swollen; distinct karyokinesis is not found. In some places the larger arteries present a focal chronic thickening of the intima which takes no part in the acute changes.

The veins are usually the seat of circumscribed or diffuse infiltration that always is connected with neighbouring foci.

There were no giant cells in this case.

Tubercle bacilli were found in fair numbers in the peri-arterial infiltration and in the walls of small arteries.

Before considering the special arterial changes in this series of cases of tuberculous meningitis a few remarks concerning the bacteriological examinations are necessary.

Of the nine cases, but one presented a mixed infection due to a staphylococcus, which was present in small numbers in the meningeal exudate and gave rise to three colonies upon the plate cultures made from the fluid.

The microscopic examination does not indicate positively that the presence of this staphylococcus had any distinctly recognisable effects upon the intensity or the character of the inflammatory changes of the case in which it was found.

Very likely the meningeal infection by this staphylococcus took place from the nose, in the mucus of which were a large number of similar staphylococci. The possibility of a general infection of the blood from some other source than the nose can not be denied in the absence of decisive investigation on this point.

That only one case of nine should show a mixed infection is a little unexpected in view of the results obtained by Babes, who examined seventeen cases of acute tuberculous meningitis bacteriologically and found, in addition to the tubercle bacillus, twice the micrococcus lanceolatus, twice the streptococcus, twice staphylococci, and in two cases two separate varieties of pathogenic bacilli, making eight of the seventeen cases instances of mixed infection.

Pasquale also found and isolated the streptococcus pyogenes in one case of tuberculous meningitis and in one case of tuberculosis of the ependyma.

In five of nine cases a superficial examination of the mucus and muco-pus of the nose for tubercle bacilli by means of the staining method gave a positive result in two instances (Cases I and VII). The indications are, then, that a more thorough search, accompanied with inoculations into susceptible animals, would have given more numerous positive results.

The tubercle bacilli present in the two instances may have found their way into the nose from the tuberculous foci present in the lungs in both instances, or through inhalation from the outside (Straus), or from the meningeal exudate in which they are present in cases of tuberculous meningitis.

Gradinego found in this disease tubercle bacilli in sections from the nerves that pass through the internal ear and also in the pharyngeal mucus in the neighbourhood of the openings of the Eustachian tube, and he considers that they pass out from the subarachnoid space by the route thus indicated.

In three cases cultures of tubercle bacilli were obtained by merely inoculating a drop or two of meningeal fluid upon glycerin agar or blood serum.

It is consequently easy to secure cultures of bacilli from the lesions of tuberculous meningitis. This is shown also by von Wunschheim, who obtained cultures three times out of five attempts by inoculating the small nodules of acute tuberculous meningitis directly on serum.

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The results of the histological examination of the instances of tuberculous meningitis that have been detailed in the foregoing pages demonstrate that in every case extensive vascular changes of various kinds were present.

The fact that in the arteries not only the adventitia, but also the media, and even the intima, present frequent and marked tuberculous lesions, should naturally attract much attention.

The arterial tuberculosis presents itself for discussion not only from the essentially histological point of view, but requires consideration also with more particular reference to the mode of the invasion of the arterial wall, whether from within or from without, and to the relation in each instance of the arterial changes to the general meningeal infection.

With these problems in sight, the essential lesions of the various cases may be summarized in this general way:

Cases I, II, III, and IV are accompanied with one or more

chronic meningo-encephalitic foci. In Case I there is a caseous nodule one cubic centimetre large along the anterior margin of the left cerebellar hemisphere, and connected with the pachymeninx; in Case II are numerous pea-sized nodules in the cortex connected with the pia, and in Case III many larger and smaller caseous masses in the brain, cerebellum, and pons; in Case IV the beginning of the Sylvian arteries and of the arteries of the corpus callosum is surrounded by chronic meningo-encephalitic masses.

In the remaining instances larger chronic, tubercular foci are not present in the brain.

In all the cases there were multiple chronic, caseous masses in various parts of the body, the peribronchial lymphatic glands, for instance, being more or less involved in every case.

In three instances (III, VI, and IX) there was a general acute, miliary tuberculosis, and two other cases (V and VIII) presented a universal subacute tuberculosis.

In Cases I and III the proliferation and degeneration, as far as the arteries are concerned, involve principally the adventitia and the media. In some places there is a diffuse affection of the whole wall, and very frequently is this the case in the smaller arteries. Both present also some subendothelial, intimal proliferation, but the changes in the outer walls are, altogether, the predominating.

In Case II the Sylvian artery presents a circumscribed infiltration that reaches the intima, and the smaller branches in the Sylvian fissures are the seat of an endarteritis without marked adventitial or medial changes.

In Case IV the entire walls of the larger basal arteries are infiltrated and caseous, so that the structure is not recognisable, and degenerated cell masses are found upon the intima that may have been miliary tubercles. Otherwise a thorough examination fails to demonstrate intimal tubercles or giant cells in any one of these four instances.

In Case V, however, the arteries of the corpus callosum present distinct intravascular tubercles with diffuse infiltration, caseation, and giant cells; extensive changes are present in the media and

the adventitia, as well as in the surrounding tissues. Small caseous masses in the pia are closely bound to the arterial walls.

In this case the gelatiniform, sero-fibrinous exudate characteristic of tuberculous meningitis was not present. Nevertheless, it does not seem quite correct to regard the process as a subacute miliary tuberculosis of the pia exclusively, because the turbid meningeal fluid contains many epithelioid cells, leucocytes, and also tubercle bacilli. The diffusion of the bacilli in the meningeal fluid may have occurred very shortly before death, and the gross inflammatory products may have been absent on this account.

In Case VI the intravascular changes are best marked (Plate VIII, Figs. 1, 2, and 4). They consist of a diffuse endarteritis that at times leads to occlusion of the lumen, especially in the smaller arteries. Giant cells and typical tubercles occur in the endarteritic districts. Isolated intimal tubercles are present. The diffuse intimal proliferation and the intimal tubercles occur as the only disease of the arterial wall as well as in association with changes of variable intensity in the other coats.

Cases VI, VIII, and IX are really identical as regards these lesions, the only differences being those of degree.

Case VII is characterized by sharply circumscribed infiltrations through the whole thickness of the arterial wall that often lead to small aneurismal bulgings (Plate VIII, Fig. 5). Only the innermost layers of this granulation tissue may show caseation. Isolated masses of cells, at times caseous, are also found upon the intima without any changes in the other walls. In addition there is present a diffuse endarteritis, at times associated with changes in the adventitia and the media, at times being the only lesion present in the whole arterial wall.

In Case VIII is an endarteritis with indistinct tubercles that show signs of caseation, associated with diffuse and extensive changes in the media, but there are also intimal tubercles with but slight alterations in the other walls, and the mode of origin of the minute granulations connected with the arteries of the pia over the convexity

can be traced with reasonable certainty to primary subendothelial proliferations in the intima which contain giant cells.

In Case IX are distinct intimal tubercles without giant cells but with caseation accompanied with a more diffuse endarteritis and slight infiltration of the media and the adventitia. In the smaller arteries are very diffuse changes.

In the sections from all the cases tubercle bacilli were present in small numbers in the intimal lesions, in the lumen of infiltrated veins and smaller arteries and their walls, and in much larger numbers in the adventitial proliferation. The infiltrated part of the Sylvian artery in Case IV contained bacilli in great numbers.

The demonstration of tubercle bacilli, free and intracellular, in the meningeal exudate in the majority of the cases has been considered.

In such cases as all of these under consideration at the present time the extensive and multiple tuberculous processes in various organs indicate clearly enough that tubercle bacilli must have gained entrance into the general circulation. This circumstance alone would be good reason for regarding the tuberculous meningitis as of a hæmatogenous origin. In addition, the extensive lesions of the arterial intima in all these cases, especially when studied in their relation to the changes in the other layers of the arterial wall, furnish direct anatomical evidence that bacilli were deposited upon the intima from the blood, and that the meningitis positively resulted from blood infection.

It is true that in the three first instances described distinct intimal tubercles with giant cells were not found, and that the changes in the adventitia and in the media of the larger arteries predominate over those in the intima, the latter consisting in the main of a more diffuse subendothelial proliferation. It might consequently be said that perhaps the chronic tuberculous masses in the brain and in the meninges, which were present, more likely furnished the source of the meningeal invasion in these cases.

In no one of the three cases, however, does the distribution of the characteristic lesions indicate that the meningitis developed from

the chronic cerebral foci. It matters not where the old caseous masses may be situated, the acute lesions, the miliary tubercles, and the exudate are found best marked around the arteries of the Sylvian fissures and of the corpus callosum. The meningeal changes do not radiate in all directions from the chronic foci, but they have that general distribution with the special predilection for the Sylvian arteries which is so characteristic of blood-borne infection.

When the intravascular changes in these cases are studied in the light in which such lesions are presented to view in the remaining instances, then it becomes plain that arteries, especially small ones, the entire wall of which is infiltrated and caseous, may just as well have been invaded from within as from without, and that an endarteritis which is not accompanied with very extensive changes in the media and the adventitia is most likely the primary arterial change. The occasional presence of tubercle bacilli in the lumen and in the wall of smaller arteries (Cases I and III) also point to infection by way of the blood.

The gross distribution of the meningeal lesions, then, the endarteritis present in all the three cases, though in varying degree, as well as the diffuse involvement of arteries, especially the smaller, afford substantial anatomical reasons for regarding the meningitis as of hæmatogenous origin, and the arterial changes presented as in part at least the direct result of this mode of invasion.

In Case IV the intense involvement of the large basal arteries in the chronic leptomeningeal process may be taken to indicate that the latter began in the arterial wall, but the local changes are in a measure too diffuse and the degeneration too far advanced to form any definite idea as to the exact order of the development of the lesions.

The situation of the chronic meningeal foci in this case, associated as they are with such diffuse arterial changes in the neighbourhood, suggests the possibility that these masses furnished at one and the same time the source for an arterial dissemination of the bacilli into the leptomeninx as well as of an infection of the pial lymph spaces.

In the remaining cases the arterial lesions indicate with great certainty that an invasion of the wall frequently occurred from within. While the wall in these cases also presents diffuse changes—changes that especially involve the adventitia, from which they may spread inward—there are also very extensive and important changes in which the intima plays the essential part (Plate VIII, Figs. 1, 2, 3, 4, and 5).

As already shown, tubercles in the intima may occur in association with more or less diffuse changes in the other coats, accompanied with an extensive endarteritis, or they may occur without any other changes of the arterial segment in the intima of which they are situated (Plate VIII, Fig. 4). Foci of granulation tissue that perforate the arterial wall more or less sharply also seem to begin in the intima (Case VII, Plate VIII, Fig. 5).

Associated with these typical tuberculous changes is an endarteritis that may occur independently or be connected with infiltration in the media and the adventitia. The presence of this endarteritis without changes in the other layers of the wall, but being confined to the internal surface of an artery throughout its entire extent,* shows conclusively that this very interesting form of intravascular lesion in tuberculous meningitis may be primary and due to inflammatory agents circulating in the blood (Plate VIII, Figs. 1, 2, and 3).

Bearing the fact in mind that such extensive and undoubtedly primary intimal lesions may be present in tuberculous meningitis, it will become exceedingly difficult, if not entirely impossible, to decide where the process may have had its point of origin in the case of more or less diffuse involvement of the arterial wall when associated with endarteritis.

On this account it would be unsafe to regard the changes of the arterial wall to proceed always from without inward, as Guarnieri concluded. Even the greater intensity and the apparently greater

* By means of the serial sections it was possible to establish the exact relations that the changes in the arterial wall may bear to each other when not too far advanced. The actual existence of an isolated intimal tubercle or of a limited focus of endarteritis, for instance, could only be demonstrated by means of serial sections of the whole extent of the artery involved.

age, as determined by degeneration, of lesions in the adventitia and the media can not be regarded as absolute criteria as to the point of primary attack of the arterial segment, because there is occasionally found a district of hyaline degeneration of the inner part of the media immediately underneath intimal foci that present but slight retrogressive changes, no other lesions of the wall being visible. Degeneration can consequently develop in the media secondarily to well-preserved adjacent foci of proliferation in the intima.

Invasion of the media and of the intima from without certainly occurs in tuberculous meningitis as well as elsewhere, as, for instance, in the lungs, and with great frequency. This is shown best by the presence of diffuse or circumscribed adventitial proliferation that gradually extends into the media and reaches the intima perhaps only at one point. In this way an endarteritis due to direct extension from without may arise and extend along in the intima.

It seems, however, that an endarteritis distinctly traceable from without rarely reaches the extent of the primary endarteritis. Intimal tubercles with giant cells were not found upon the arteries in which it could be shown with certainty that the process invaded the arterial wall from without. In connection with this, attention is called to the fact that in many cases the only giant cells found after vigilant search were those in the endarteritic foci.

Whether or not tuberculous processes confined to the adventitia can give rise to an acute intimal proliferation, without direct extension of the inflammation through the media, hardly needs discussion. Chronic inflammatory processes in the neighbourhood of arteries are often stated to call forth an obliterating endarteritis (see note, page 119). In acute tuberculous meningitis one very often observes extensive changes in the adventitia associated with proliferative changes of variable extent in the intima, the media remaining unchanged. Whether the changes in the outer and the inner layers bear any direct and intimate relations to each other under these circumstances must be left undecided for this one reason, if for no other—namely, the impossibility of excluding a direct hæmatogenous origin of the endarteritis. The same consideration applies to the obliterating,

subendothelial intimal proliferation observed by Weintraud in more chronic meningo-encephalitic tuberculous foci, and attributed by him, in accordance with the views of many writers, to influences acting upon the intima from without; the intimal lesions may have been caused by direct infection from the blood.

It is self-evident that following the total invasion of an arterial wall from without, a local diffusion of bacilli might occur in the corresponding branches with, perhaps, a secondary endarteritis due to implantation of the bacilli upon the intima. And of course the same manner of propagation may follow primary intimal foci. In either case a destruction of the endothelium would be most favourable for the admission of bacilli into the blood current, and at such times thrombosis may hinder the process from spreading in this manner.

The tuberculous endarteritis that has been described as occurring primarily and independently of changes in the outer arterial coats demonstrates that tubercle bacilli are implanted in the intima. The intact endothelium usually present shows that the bacilli must reach the subendothelial layer at once after passing through the endothelial lining.

Attention may be called to the fact that the arteries in the Sylvian fissures present probably the greatest degree of endarteritis. This fact may be accounted for by the favourable conditions offered by these vessels for the localization of the microbes. In other words, it would seem that to some extent the same conditions obtain in the localization of the bacilli as are so frequently observed to be operative in connection with the import of emboli into the brain.

The marked degree of primary endarteritis in the Sylvian arteries explains also, to some extent at any rate, the relatively greater development of the adventitial and extravascular changes in the Sylvian fissures than elsewhere.

The histological examinations show that the muscular coat of the large as well as the small arteries is the frequent seat of infiltration and caseation that may have their origin in the adventitia or in the intima, according as the invasion occurs from within or from without. Appearances indicating the origin of new cells from the muscle

fibres, which Rindfleisch is inclined to believe that he has observed, were not present in any of these instances, in all of which the medial changes seemed to be the result of a direct extension of the process in the adventitia or in the intima.

When the infiltration invades the media from the intima, the elastic membrane always presents evidences of a more or less direct destruction of its continuity (Plate VIII, Figs. 4 and 5). At times it is split up by invading cells that seem to be in the act of passing through it. When the process is further advanced it may present a sharply cut defect occupied by granulation tissue or by caseous material. At other times the elastic membrane as well as the inner part of the media undergo a peculiar hyaline degeneration underneath foci of proliferation in the intima. In Case VII, in which the arterial walls present numerous aneurismal bulgings at the seat of perforating foci of tuberculous infiltration, the ends of the perforated elastic layer are bent outward.

In the descriptions of the microscopical changes frequent reference is made to the infiltration and caseation of the whole wall, especially in smaller arteries, and to a more or less extensive hyaline degeneration whose favourite seat seems to be the media, but which may occur in any part of the vessel wall as well as in the extravascular infiltration. This hyaline degeneration was observed in the media as the only change in the whole wall in addition to focal or diffuse endarteritis.

This peculiar hyaline necrosis in tuberculous meningitis has been fully described by Guarnieri, according to whose opinion the hyaline substance represents the end products of degeneration in the tubercle cells, the muscle cells, and the exudate.

The pial arteries in tuberculous meningitis consequently present excellent opportunities for the study of hyaline degeneration which here seems to appear very rapidly under the influences of the acute inflammatory process.

In addition to the arterial changes, those in the veins merit attention (Plate VIII, Fig. 6). In all the instances the veins, large and small, show diffuse or circumscribed infiltrations of the walls that

invariably proceed from changes in the neighbourhood, as, for instance, in an adjacent artery. Giant cells frequently occur in the infiltrated walls often lying next to the blood stream. Caseation and disintegration are also found, and frequently the lumen is thrombotic. Tubercle bacilli are also present in the infiltrated walls.

No doubt general miliary tuberculosis may develop secondarily to tuberculous meningitis on account of a dissemination of tubercle bacilli from these extensive and constant lesions of the pial veins. Tedeschi found that a general infection occurs very rapidly after the subdural inoculation of rabbits and other animals with tuberculosis.

All authors emphasize the prominent part played by the adventitia in the formation of the tubercles and more diffuse infiltrations in tuberculous meningitis. It is in the lymph spaces of this layer of the arterial wall that the tubercle bacilli appear to find the most favourable conditions for rapid growth. Especially in the early part of the process large cells, often containing tubercle bacilli can be seen filling the adventitial lymph spaces. Some of these cells may be carried away with the lymph stream, and, as the cells fall to pieces, the bacilli are set free to start new centres of proliferation. Thus in Case V the presence of many bacilli in the epithelioid cells of the subarachnoid fluid, and also in similar cells in the lymph spaces about the arterial tubercles, shows most conclusively the manner in which the bacilli may be diffused in the subarachnoid space from adventitial foci.

It will, of course, be recollected that the lymph spaces of the adventitia of the arteries can be injected from the subarachnoid space (Stöhr).

Regarding the further evolution of the lesions of tuberculous meningitis, it may be said that the accumulation of exudate containing bacilli in the large lymph cisterns of Key and Retzius, in the Sylvian fissures, and at the base, is certainly most favourable for the development of extensive extravascular lesions in these localities.

The spread of the bacilli from the adventitial lymph spaces throughout the subarachnoid effusion may lead to a spinal leptomeningitis. The latter was present in four out of the five of these cases

in which the spinal cord was examined—namely, in Cases I, III, IV, and VII—and in all instances the arterial lesions pointed to a lymphogenous invasion. An infection of the spinal pia through the blood is, of course, possible, but in our cases the arterial lesions do not indicate that such was the case.

The cerebro-spinal fluid may also carry the bacilli into the lymph spaces of cranial as well as of spinal nerves, where they may cause an interstitial (tuberculous) neuritis, as shown by Schultze with reference to spinal nerves, by Kahler in case of the oculo-motor nerve, by Gradinego as regards the nerves that pass through the middle ear, and by Deutschmann in case of the optic nerves in rabbits, in which he produced experimental tuberculosis of the brain and its membranes.

From the subarachnoid space the bacilli may also find their way into the nose and the pharynx, as already pointed out.

The relation of the changes in the intima observed in these cases to the changes in the other coats has been explained. It has been emphasized that the intimal changes are of comparatively slight degree, and without definite tubercles or giant cells, in those places where it can be definitely shown that the invasion of the artery occurs from without; that in the case of total infiltration and caseation of an arterial segment no opinion can be formed as to the order of the development of the lesions; that intimal proliferation, often associated with giant cells, distinct tubercles, and caseation, and without extensive confusing changes in the media and the adventitia, is positive anatomical evidence of the implantation of bacilli (which may be present in these lesions) from the blood.

As already pointed out, Huguenin, Huettenbrenner, Cornil, and Hirschberg have also described similar intravascular tuberculous lesions in the pia, and have expressed practically the same views as the preceding concerning the signification of such lesions as regards the mode of infection.

Tuberculous changes in the intima which are susceptible of the sole explanation that they are the direct result of the import of the bacilli by the blood, were found more or less clearly marked in all the nine cases of this series, and in some cases—as, for instance, in

Case VI—the intravascular changes are almost as extensive and as pronounced as the extravascular.

The general conclusion is consequently warranted that in tuberculous meningitis of blood-borne infection an unexpectedly frequent and significant localization of the bacilli occurs upon the intima of pial arteries in addition to that in the arterial adventitia.

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Experimental tuberculous meningitis has been studied by Deutschmann, who investigated the relation between miliary tuberculosis of the brain and its membranes and affections of the eyes after injecting tuberculous material underneath the skull of young rabbits; by Daremberg, who produced tuberculous meningitis by the injection of pure cultures underneath the skull of rabbits; and by Tedeschi, who injected tuberculous material into the cranial cavity and spinal canal of eighty-five animals of various kinds. In addition to producing a meningo-myelitis and a meningo-encephalitis as well as diffuse miliary tuberculosis, the last-named writer found that the inoculation of tubercle bacilli into the nervous centres of animals resulted in a marked increase of virulence on the part of the bacillus. This virulence became normal again after passing the bacilli through the body of guinea pigs.

Inasmuch as there were no experiments in which the attempt had been made to produce tuberculous meningitis in the manner in which the disease nearly always develops in man—namely, through arterial infection—a series of experiments with this object in view were commenced. These investigations are as yet incomplete; but as the results already obtained in a measure bear upon the conclusions expressed as regards the tuberculous endarteritis that has been described, the report of the following experiment may be introduced:

May 16, 1895. Injection of 0·1 cubic centimetre of a bouillon suspension of a glycerin-agar culture of tubercle bacilli into the left carotid artery of a large rabbit.

The carotid was ligated above and below the point of puncture.

Soon after the operation the animal began to emaciate. Shortly

before it was killed the media of the left eye became turbid; there was some pericorneal injection and turbid conjunctival secretion.

June 5th, 6 P. M., killed.

Section.—Pronounced emaciation. The media of the left eye turbid. There is no increase in the amount of cerebro-spinal fluid, and the pia mater is everywhere transparent and thin. Along the vessels of the convexity of the left hemisphere, especially over the posterior half, are numerous minute grayish nodules of varying size, the largest being perhaps one millimetre in diameter. There are no visible nodules in the pia over the right hemisphere, over the cerebellum, the bulb, or the base of the left hemisphere.

There are numerous miliary nodules in the liver and some in the spleen, but not in any other organs. The left carotid, being cut across between the two ligatures a little above the clavicle, shows that the ends are filled with a whitish granular mass. Immediately above the upper ligature the vessel is surrounded by a flat, oval caseous mass one centimetre in its greatest diameter.

Microscopic Examination.—After fixing in sublimate and staining on the slide with hæmatoxylin and carbol-fuchsin, sections through the meningeal nodules and the subjacent cortex show a dense infiltration of lymphoid cells around small granular masses, containing tubercle bacilli, which seem to occupy the interior of small arteries, the walls of which are entirely converted into granulation tissue. Sometimes the granular mass, including the bacilli, becomes infiltrated with nuclei and assumes an appearance not unlike giant cells. At the periphery of such nodules the lymph spaces are loosely filled with cells, but bacilli are seldom found except in the central part of the tubercles.

The interior of the left hemisphere contains a large number of minute foci of granulation tissue in which may be giant cells and tubercle bacilli, the latter embedded in granular masses.

The nodules in the liver are miliary tubercles. The ciliary body of the left eye contains miliary tubercles with giant cells located in the vicinity and about the arteries. The anterior and posterior chambers are filled with granular material.

This experiment shows that a meningeal and cerebral miliary tuberculosis can be produced in rabbits by injecting bacilli into the carotid—i. e., through direct hæmatogenous infection. The resulting tubercles develop in connection with the smaller arteries. In the experiment described the lesions were too far advanced to afford any definite observations as to the early histogenesis of the process.

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In the foregoing has been described a tuberculous endarteritis the histogenesis of which presents several points of interest.

The histological appearances may be briefly recapitulated as follows: In addition to intimal tubercles there occurs a more or less extensive endarteritis, which reaches its greatest degree of development in the medium-sized or smaller arteries in which it frequently leads to great narrowing and almost complete obliteration of the lumen. That this endarteritis may be primary and not due to extension from the outside is readily shown by serial sections extending through endarteritic foci in which the arterial wall is otherwise normal (Plate VIII, Figs. 1, 2, 3, and 4).

In all well-preserved specimens the proliferation—which consists of epithelioid cells with but occasional lymphocytes and polynuclear leucocytes and, quite rarely, multinucleated giant cells (Plate VIII, Fig. 2)—is invariably subendothelial, the cell layers lying between the elastic lamina and the intact but loosened and elevated endothelium (Plate VIII, Figs. 1, 2, and 3). Frequently characteristic intimal tubercles develop in this proliferation, and as they become caseous the endothelium over them may disappear (Plate VIII, Fig. 4).

In the early stages the endarteritis presents itself in the form of small nodular accumulations of cells that present unmistakable signs of active proliferation underneath an intact endothelium, which is lifted up into folds. In many places the subendothelial cells penetrate the elastic membrane and invade the muscular coat to a greater or less extent.

Similar appearances, though less marked, are found in arteries that are invaded by cell infiltration from the adventitia.

The subendothelial intimal proliferation may present caseation

and hyaline degeneration. Only occasional tubercle bacilli are found in and among the epithelioid and other cells.

The endothelial cells remain entirely passive and present no appearance of active proliferation, the lining being mechanically lifted away, as it were, by the increasing cell accumulation underneath. At times the endothelial lining becomes disarranged, especially when the endarteritis is very marked.

The origin of the epithelioid cells that in the main constitute the subendothelial proliferation is a question of great interest.

Nearly all the authors that have described endarteritic changes in tuberculous meningitis refer to these cells as the results of multiplication on the part of the endothelium. Huguenin, Guarnieri, Brodowski, Baumgarten, Hirschberg—all seem to take this view.

Baumgarten, in his classical research upon the histogenesis of tubercle, also refers to certain changes in the intima in experimental pulmonary tubercle as due to endothelial proliferation.

Metschnikoff assigns to the endothelial cells an active rôle in the formation of hepatic and pulmonary tubercle.

This is not the place to enter into a detailed discussion of the origin of epithelioid cells in general. The views of Baumgarten and of Metschnikoff are familiar enough. The recent studies by Kostenitsch and Wolkow and by Borel of the development of experimental tubercle treat of the various phases of this question with considerable minuteness of detail.

While it is evident that the morphology of the epithelioid cells in the endarteritis of tuberculous meningitis can not explain their nature or declare their derivation, it seems that their origin from the endothelial cells can be positively excluded on account of the condition of the endothelial lining itself. Were the epithelioid cells in question produced by proliferation of the endothelial cells, then the endothelial lining would be disarranged, and the subendothelial proliferation would no longer be limited on the side of the lumen by a single layer of typical endothelial cells that show no signs of multiplication whatsoever. The division of a single endothelial cell into two would certainly result in some displacement of the dividing cell

with reference to the other cells in the endothelial lining. Baumgarten's figure of a young metastatic tubercle in the lung shows, on the other hand, like the sections of the tuberculous endarteritis, an artery with a mass of epithelioid cells between an unbroken and unchanged row of single and typical endothelial cells and the elastic layer. Near by is represented karyokinesis in what may be the unchanged endothelium, but the karyokinetic figure may also be in a subendothelial connective-tissue cell.

For these reasons it may be concluded that the endothelial cells take no active part in the production of epithelioid cells in tuberculous endarteritis; on the other hand, it would seem that the endothelial cells remain not only passive, but even resistant to the bacilli and their products, because the lining remains intact and unbroken until the proliferation almost fills the entire lumen. When caseation occurs, however, then the endothelial cells disappear.

As already stated, the bacilli must apparently pass through the endothelium and into the subendothelial layer of connective tissue, which, from the early appearances of the endarteritic proliferation, most likely gives rise to the epithelioid cells.

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Conclusions.

1. In tuberculous meningitis there is a tuberculous endarteritis characterized by the formation of intimal tubercles and a diffuse subendothelial, intimal proliferation due to implantation of tubercle bacilli from the blood. From the endarteritis the infiltration may spread into the muscular coat and the adventitia, and the whole wall may undergo caseous and hyaline degeneration.

2. Tuberculous proliferation in the adventitia may invade the media and the intima, and the whole wall of the arterial segment may undergo degeneration.

3. The veins are constantly the seat of more or less extensive infiltration, which always results from adjacent extravascular or arterial foci.

4. The epithelioid cells of the subendothelial, tuberculous intimal

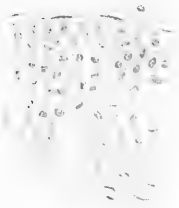

FIG. 1

FIG. 2

FIG. 3

FIG. 4.

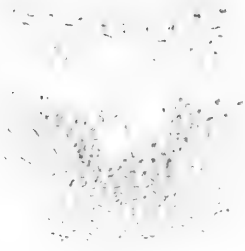

FIG. 5

FIG. 6

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proliferation are most likely derived from the subendothelial layer of connective tissue and not from the endothelial lining.

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There remains the pleasant duty of expressing the warmest thanks to Prof. Chiari for his constant aid and advice during the progress of the work embodied in this article.

Description of Plate VIII.

Fig. 1.—Subendothelial proliferation of epithelioid cells without any changes in the other coats. Note the intact endothelium. From Case VI. Zeiss, Obj. E, Oc. 4.

Fig. 2.—Subendothelial intimal proliferation with multinucleated giant cell. The media and the adventitia are normal, and the endothelium is intact and unchanged. From Case VI. Zeiss, Obj. E, Oc. 4.

Fig. 3.—Subendothelial proliferation leading to almost complete occlusion of the lumen of a medium-sized artery without any changes in the muscular or adventitial layers, the endothelial lining being visible in the centre of the vessel. From Case IX. Zeiss, Obj. A, Oc. 4.

Fig. 4.—Diffuse endarteritis with small intimal tubercle; at *a*, multinucleated giant cell; underneath the tubercle there is perforation of the elastic lamina and beginning slight infiltration into the media; otherwise the media and the adventitia are quite unchanged. The endothelium, which is present over the diffuse intimal proliferation, is absent over the tubercle, which is undergoing caseation and covered with a finely granular thrombotic mass. From Case VI. Zeiss, Obj. A, Oc. 2.

Fig. 5.—Caseous intimal focus which has caused perforation of the elastic layer, the ends of which are bent outward, and infiltration into the media and the adventitia, the whole having a slight resemblance to a small aneurism. Note the commencing subendothelial intimal proliferation in the opposite wall of the artery. From Case VII. Zeiss, Obj. E, Oc. 2.

Fig. 6.—Focal tuberculous infiltration with giant cell into and through the wall of a vein with secondary thrombosis, the process being due to extension from the neighbourhood. Case II. Zeiss, Obj. C, Oc. 4.

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THE PRODUCTION OF DIPHTHERIA TOXIN.

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The production of diphtheria antitoxin was undertaken by the New York City Health Department in September, 1894. The number of horses, at first limited to a few, was gradually increased until in January, 1895, forty were under treatment. In order to supply sufficient toxin for the injection of these horses it had to be produced in large quantities. It was also desirable to obtain the toxin in as concentrated a form as possible, so that the horses, which required ever-increasing amounts, should not receive subcutaneously too large quantities of fluid. The statements of Roux and Behring were so explicit as to the methods of producing toxin that their advice was followed for a time without questioning. At considerable expense and trouble virulent diphtheria bacilli were either grown for several weeks in broth contained in especially constructed flasks and under a constant current of fresh air, as advised by Roux, or the bouillon cultures contained in Erlenmeyer flasks were allowed to stand in the incubator at 35° for from four to six weeks, as recommended by Behring.

The results were not satisfactory. The toxin produced by these methods had generally a strength of which 0·1 cubic centimetre would kill a 250-gramme guinea-pig in four to five days (Behring's normal toxin). Occasionally 0·05 cubic centimetre would kill, while, on the other hand, not infrequently 0·2 cubic centimetre or even more was required.

The moderate strength of the toxin obtained with abundant growth of bacilli, and the fact that the toxin was never found to increase at later periods in cultures after they had developed for three weeks at

35° C., but rather was frequently found to slowly disappear when left longer in the incubator, made us resolve to thoroughly investigate in a practical way the length of time needed for the production of toxin and the best conditions, both as to culture and media, under which to obtain it. The rapid production of very poisonous toxin in human and experimental diphtheria suggested to us that under favourable conditions it would probably be as quickly produced by very virulent bacilli in bouillon.

The experimental work described in this paper was done in the Bacteriological Laboratory of the Health Department of the City of New York, of which Dr. Hermann M. Biggs is the director, and comprises two series of investigations. The first, begun in June, was terminated in the latter part of July; the second, begun in October, is still unfinished.

Technique.

The bouillon in each set of experiments, after being prepared from meat in the usual way, was first poured into a single vessel and made neutral to litmus. From this common stock portions were removed, and, after having added the amounts of normal soda solution and peptone desired, were poured into 600-cubic-centimetre Erlenmeyer flasks. All the flasks contained the same amount of bouillon—250 cubic centimetres—and were similarly stoppered with cotton. In the second series of experiments each flask was covered by a paper cap as an additional precaution to prevent contamination. All the flasks were placed in the same incubator and kept at a temperature of 35° C. By these means all the conditions for the growth of the different cultures were exactly similar except those which we purposely made different, such as the amounts of peptone and of alkali, and the degrees of virulence of the different bacilli used. The inoculation of the bouillon in all the flasks of each series with the diphtheria bacilli was made from a single bouillon culture of forty-eight hours' growth. Each flask was inoculated with an equal amount—2·5 cubic centimetres—of this thoroughly shaken culture.

In order to test the influence of the reaction, of the percentage of peptone, and of the degree of virulence possessed by the bacillus upon the production of toxin, the following scheme was carried out. The details of the second experiment, as being the most extensive, will be first described, and then later the points in which the first one differed from it. The bouillon used contained only faint traces of glucose derived from the meat. After being mixed thoroughly it was divided into three portions. To one half of the bouillon one per cent peptone, to one third two per cent peptone, and to one sixth four per cent peptone was added. Each one of the three portions of neutral bouillon was then further subdivided into four equal parts. The first part was left neutral, to the second five cubic centimetres, to the third ten cubic centimetres, and to the fourth fifteen cubic centimetres of normal soda solution (forty grammes of sodium hydrate to the litre) were added. With these different varieties of bouillon seventy-two 600-cubic-centimetre Erlenmeyer flasks were partly filled, each flask receiving two hundred and fifty cubic centimetres. These flasks, after being both plugged with cotton and covered with loosely fitting paper caps, were sterilized on three successive days for one hour each in Arnold steam sterlizers.

The flasks were now finally divided into three groups, each of which contained an equal number of all the different varieties of bouillon. For the inoculation three cultures were employed which differed somewhat in virulence. The one named 8, which was obtained two months previously from a very mild case of diphtheria, killed a 500-gramme guinea-pig in a dose of 0·002 cubic centimetre. Culture named R came from a moderately severe diphtheria, while J was from a rapidly fatal case. These two were equally virulent, killing the same-sized guinea-pigs in doses of about 0·01 cubic centimetre. None of these cultures had been passed through the bodies of guinea-pigs, being merely transplanted from the pellicle into fresh bouillon every two days. The flasks of each division were inoculated on October 27th with 2·5 cubic centimetres of a forty-eight hours' growth from a single flask of one of the three cultures. The

flasks were then all placed in the same incubator and kept at a temperature of 35° C.

After twenty-four hours' growth in the thermostat a measured quantity was withdrawn from each flask, after its contents had been shaken, by means of sterilized pipettes and placed in sterile glass bottles; after the addition of 0·5 per cent of carbolic acid, these bottles were stored in the ice chest. From a certain number of the flasks larger quantities were withdrawn and immediately filtered. To the filtered culture fluid, after testing its sterility, 0·5 per cent carbolic acid was also added.

At the time that the fluid was removed from each flask agar plates were made from each culture in order to test its purity. From eight cultures the agar plates were made with a tube of fluid agar to which a loop full of the well-shaken culture had been added. The colonies which developed were then counted after forty hours' growth at 35° C.

Similar drawings were made from all of the seventy-two flasks upon each of the first days, and then at longer intervals up to the seventy-fifth day.

If from time to time one or more flasks became contaminated they were withdrawn. We were, however, very fortunate in this respect in this second experiment. We believe this to be partly due to the protection of the cotton plugs from dust by the paper caps.

The first series of experiments differed only so far as the bouillon was concerned, in that we added greater amounts of alkali to some of the flasks and did not use as great amounts of peptone in others. The two cultures were H and P, each killing 500-gramme guinea-pigs in doses of 0·025 cubic centimetre. In this experiment we commenced to withdraw the culture fluid on the tenth day, and did not immediately filter any of the test samples. As the details are somewhat intricate, they are summarized in the following table:

TABLE I.

Showing the Number of Flasks employed, the Amount of Alkali added, and the Cultures used in the Experiments.

First experiment, 30 flasks.		Second experiment, 72 flasks.	
One per cent peptone. Two thirds of flasks inoculated with culture P and one third with culture H.	Neutral, 3 flasks. 5 c. c., " " 10 " " " 20 " " "	One per cent peptone. 36 flasks divided equally between the 3 cultures, 8, R, and J.	Neutral, 9 flasks. 5 c. c., " " 10 " " " 15 " " "
Two per cent peptone. One flask of each lot inoculated with H, the others with P.	30 " " " Neutral. " " 5 c. c. " " 10 " " " 15 " " " 25 " " "	Two per cent peptone. 24 flasks divided equally between the 3 cultures.	Neutral, 6 " 5 c. c., " " 10 " " " 15 " " "
		Four per cent peptone. 12 flasks divided equally between the 3 cultures.	Neutral, 3 " 5 c. c., " " 10 " " " 15 " " "

Neutral = neutral to litmus.

5, 10, 15 c. c. = 5, 10 and 15 cubic centimetres of normal sodium-hydrate solution added to each litre of neutral bouillon.

The Bacillus 8 killed 500-grm. guinea-pigs in 0·002 c. c. dose in 7 days.

"	R	"	"	"	" 0·01	"	"	" 2	"	0·005 c. c.	not fatal.
"	J	"	"	"	" 0·01	"	"	" 2	"	0·005	" "
"	P	"	"	"	" 0·025	"	"	" 2	"	0·01	" "
"	H	"	"	"	" 0·025	"	"	" 2	"	0·01	" "

THE PERIOD AT WHICH THE DIPHTHERIA BACILLI BEGIN TO PRODUCE TOXIN IN APPRECIABLE AMOUNT, AND AT WHICH THE TOXIN HAS ACCUMULATED TO THE GREATEST EXTENT.

The work of Roux and Yersin * upon this subject is not only the earliest, but also the most extensive. With but minor additions it comprises practically all that has been published upon the subject. Their results as contained in four reports may be summarized as follows:

If a flask of slightly alkaline bouillon is inoculated with a quantity of virulent bacilli and kept at a temperature of about 37° C., the bacilli rapidly proliferate and produce certain changes in the broth. The reaction of the broth becomes acid in a few days, and then again in a variable length of time alkaline. These changes require under the ordinary methods of growth three and four weeks, but when the bacilli grow in broth to which a very free access of air is permitted

* Roux. *Annales de l'Institut Pasteur*, 1888, 1889, 1890, 1894.

they may take place within two weeks. Roux and Yersin state positively that strong toxin is not produced by the bacilli in less than three weeks in broth cultures grown in the usual manner, and in those to which a free access of oxygen is supplied by the constant air current in not less than two weeks. They also assert that strong toxin is never produced while the reaction is still acid.

So far as we can determine, the essential portion of their statement that strong toxin is produced only after the lapse of a number of days and after the alkaline reaction has been established has been accepted by all German and other investigators. Some, indeed, have discovered that toxin is produced at times somewhat earlier than Roux mentions. Thus Spronck and van Furenhout,* as shown in their report, have recently obtained strong toxin from a very virulent bacillus within thirteen days in a culture grown under the ordinary conditions, and Aronson † states that by a special method —that of inoculating the bouillon from the pellicle of a forty-eight-hour culture of virulent bacilli—he obtained very strong toxin after eight days.

The table on page 170 gives the results of our tests of the amount of toxin present in samples of broth withdrawn from different cultures at different periods of growth.

The results tabulated there show that the most virulent bacillus used by us (No. 8) produced appreciable toxin within four hours and strong toxin within twenty-four hours, 0·1 cubic centimetre killing a 324-gramme guinea-pig in three days. Moreover, this toxin was produced while the bouillon was of an acid reaction (see table VII).

By the fifth day the amount of toxin in this sample of bouillon reached its maximum, 0·005 cubic centimetres killing a 430-gramme guinea-pig in two and a half days, and then remained practically stationary, having diminished but little as late as the fifty-sixth day. The other two cultures produced fairly strong toxin in four days, and here also it reached its maximum between the fifth and seventh

* Spronck. *Annales de l'Institut Pasteur*, October 25, 1895.

† Hans Aronson. *Berichte der Pharmeutischen Gesellschaft*, May 2, 1895.

TABLE II.

Time of Production of Toxin.

Length of time of the growth of the bacilli in the culture before the withdrawal of the sample tested.	CULTURES.														
	No. 8. 2 per cent.* 5 c. c.†			No. 8². Filtrate. 1 per cent.* 5 c. c.†			R. 1 per cent.* 0 c. c.†			P. 2 per cent.* 10 c. c.†			H. 1 per cent.* 0 c. c †		
	Weight of guinea-pigs in gms.	Amount inoculated, c. c.	Result of inoculation	Weight of guinea-pigs in gms.	Amount inoculated, c. c.	Result of inoculation	Weight of guinea-pigs in gms.	Am't inoculated, c. c.	Result of inoculation.	Weight of guinea-pigs in gms.	Am't inoculated, c. c.	Result of inoculation.	Weight of guinea-pigs in gms.	Am't inoculated, c. c.	Result of inoculation.
4 hours.‡	...	.....	.....	300	2	D. in 2½ d.									
1 day.	324	0·1	D. in 3 d'ys.	403	0·025	" 4 "	415	1·00	No react'n.						
2 days.	505	0·01	" 24 "												
" "	548	0·1	" 40 h.	470	0·025	" 40 hrs.	480	1·00	"						
3 "	492	0·025	" 2 d'ys.	545	0·025	" 2 d'ys.	396	0·25	D. in 2½ d.						
4 "	495	0·01	" 2½ "	...	.....	.....	483	0·25	" 3 d'ys.						
5 "	480	0·005	" 2½ "	560	0·0125	D. in 2 d'ys.	390	0·10	" 2½ "						
7 "	530	0·01	" 40 h.	451	0·005	" 3 "	438	0·10	" 4 "						
9 "	529	0·005	" 8½ d.	...	.....	.....	335	0·10	" 4 "						
10 "	...	.....	.....	...	.....	.....	...	....	.....	409	0·25	D. in 40 h.	322	0·25	D. in 40 h.
11 "	440	0·01	D. in 2½ d.	421	0·01	No react'n.									
" "	...	.....	.....	312	0·025	Slight infiltration.									
17 "	520	0·005	Induration.												
18 "	...	.....	.....	...	.....	.....	...	....	....	395	0·25	" 24 "	393	0·25	" 60 "
25 "	490	0·01	D. in 2½ d.												
26 "	...	.....	.....	...	.....	.....	...	....	.....	421	0·25	" 40 "	306	0·25	No react'n.
35 "	380	0·01	D. in 5½ d.	367	0·05	No react'n.	385	0·10	Considerable infiltration.						
40 "	...	.....	.....	...	.....	.....	...	....	.....	477	0·25	Induration.			
56 "	340	0·01	D. in 2½ d.	...	.....	... .	290	0·10	No react.#	458	0·25	No react'n.			

* Percentage of peptone † Amount of normal soda solution added to each litre of neutral bouillon.

‡ This culture was grown in a different bouillon, but inoculation was made with same bacillus by means of a platinum loopful of the pellicle.

This culture was slightly contaminated.

days. These same results have been repeated whenever the bouillon was equally suitable for a vigorous growth of the bacillus and for the quick production of toxin.

During the past three months bouillon made from fourteen different lots of beef has been used. In all but three the development of the toxin has been about as rapid as that in the experiments given in the table. The growth of the bacilli and the production of toxin in one lot were extremely interesting, being similar to the classical description of Roux and Yersin. During the first day the bacilli grew vigorously and a fairly thick pellicle formed. At the end of twenty-four hours the original alkaline reaction changed to slightly acid, the growth of the bacilli lessened, and the pellicle partially sank to the bottom of the flask and did not immediately reform. These conditions remained up to the twelfth day, when the acidity began to diminish. On the sixteenth day the pellicle had reformed; the bouillon was turbid and alkaline. No toxin was present on the twelfth day; strong toxin on the eighteenth. In the two other samples part of the cultures remained acid, and no toxin was produced. In one the growth was very scanty. But two of fourteen samples of non-putrid meat, therefore, were unfit for the purpose of producing strong toxin.

In view of the facts brought out in the preceding experiments, the positive statements of Roux, Behring, and many others that strong diphtheria toxin is never produced until after the culture has grown for several weeks are certainly very surprising.

The explanation seems to us to be that Roux and Yersin must have had in their first experiments bouillon similar to the three lots last mentioned. Getting uniformly no toxin until the third or fourth week in this variety of bouillon, they came to the conclusion that the bacilli under no conditions produced it earlier. All bacteriologists who have been interested in the production of diphtheria toxin since then seem to have accepted to a very great degree their opinions without themselves thoroughly investigating whether they were in fact correct.

The gradual disappearance of toxin in some of the older cultures

is well shown in the table in cultures P and H, where fairly strong toxin present in the cultures on the sixteenth day had almost disappeared upon the fifty-sixth and twenty-sixth day respectively, while in cultures 8^2 and J the toxin present on the fourth day had appreciably diminished in some flasks on the eleventh and eighteenth days. We had even a more striking experience with some cultures grown under a constant current of air; strong toxin was present on the seventh day and entirely absent on the fourteenth day.

The Relation between the Activity of the Growth of the Bacilli and the Amount of Toxin produced.

In all of the cultures in which strong toxin developed quickly there was a rapid and abundant growth of the bacillus, with the formation of a more or less thick pellicle within the first thirty-six hours. An abundant growth of the bacilli did not, however, necessarily indicate the production of strong toxin. In the broth to which fifteen cubic centimetres of normal NaOH solution was added per litre, and which generally produced very little toxin, we have noticed the same abundant and rapid growth.

In the broth to which twenty cubic centimetres and thirty cubic centimetres of normal NaOH solution was added per litre, and in which practically no toxin was produced, the growth was slow at first, but later nearly equalled that in the broth to which less alkali was added. In all of the broths which have remained acid for some time and which have produced toxin of some strength only after the reaction has become alkaline, the growth during the stage of acid reaction has been somewhat scanty, the pellicle, if formed at all, being slight.

The Relation between the Number of Living Bacilli in a Culture and the Production of Toxin.

This was sought to be determined in the following way: Eight of the flasks containing the cultures (four with one per cent peptone and four with two per cent, each lot comprising all four degrees of alkalinity), from which samples were being daily drawn as described

earlier in this paper, were selected for examination as to the number of living bacilli in each cubic centimetre of broth at different periods of growth. The different alkalinities and proportions of peptone gave nearly similar results. The method employed was as follows: The culture fluid in a flask in which the living bacilli were to be counted was first thoroughly shaken and then $\frac{1}{4\,5\,0}$ cubic centimetre was removed by means of a platinum loop. This was inoculated into a tube of sterilized fluid agar cooled down to 40° C., and the whole thoroughly shaken and poured into a Petri dish. The colonies were counted at the end of forty hours' growth in the incubator. The results are shown in the following table:

TABLE III.

Showing Average Number of Colonies of Bacilli in One Field of Microscope at Different Days.

PEPTONE AND ALKALINITY.	24 hours.	48 hours.	72 hours.	4 days.	5 days.	7 days.	9 days.	11 days.
R—2 per cent, neutral.....	18	30	43	25	42	16	$1\frac{1}{9}$	$4\frac{1}{9}$
1 " 5 c. c.....	32	47	45	27	18	10	1	8
1 " 10 "	60	42	25	22	31	3	$1\frac{1}{3}$	$1\frac{1}{3}$
1 " 15 "	47	57	40	56	60	13	$1\frac{2}{3}$	$9\frac{2}{3}$

In this trial, therefore, the number of living bacilli reached its highest total at the end of forty-eight hours. From the third to the fifth day the number of living bacilli, as shown by the plates, remained nearly the same. Thereafter it rapidly diminished, until at the ninth day it was but one thirtieth of its former number. Then for a time a moderate increase occurred, the number finally falling again two weeks later. The toxin increased rapidly while there were numerous living bacilli present in the culture, and then when they decreased the toxin ceased to accumulate rapidly, and even in some cases gradually diminished. Here, if new toxin was still being produced, that already present must have been even more rapidly destroyed.

In two cultures in which the acid reaction occurred early and persisted, the number of bacilli never rose above one third of the number in the above test or in other tests where similar broth was used.

The Amount of Toxin contained within the Bodies of the Bacilli as compared with that held in Solution in the Bouillon at Different Periods in the Growth of the Culture.

In endeavouring to solve this problem the following experiments were made: First, from two of the cultures—one very virulent (8), the other somewhat less so (R)—a portion was removed each day after they had been thoroughly shaken and immediately filtered. To the filtrate, after its sterility had been proved, was added 0·5 per cent carbolic acid. The filtrate was found to contain as much toxin as the unfiltered portion of the cultures removed on the same day (see Table IV).

Second, from the quantities removed at different periods in the growth of the culture the bacilli left on the filter, after the passage of the culture fluid and of considerable quantities of sterile water, were scraped off and allowed to soak in a 0·5-per-cent alkaline carbolic solution for one week. The quantity of water was equal to the amount of culture filtered. The watery extract alone and the watery extract together with the dead bacilli were then injected into guinea-pigs. Whether the filtrate contained or did not contain very strong toxin, neither the water in which the bacilli had remained nor the water plus the dead bacilli was sufficiently toxic to produce any marked reaction in 500-gramme

Table IV.

Showing Relative Strength of Filtrate and Carbolized Culture.

Culture used.	One day's growth.			Two days' growth.			Three days' growth.			Seven days' growth.		
R–1 per cent peptone, neutral reaction.	Weight of guinea-pig in gms.	Am't injected in c. c.	Result.	Weight of guinea-pig in gms.	Am't injected in c. c.	Result.	Weight of guinea-pig in gms.	Am't injected in c. c.	Result.	Weight of guinea-pig in gms.	Am't injected in c. c.	Result.
Carbolized Culture.....	415	1·00	No reaction.	480	1·00	No reaction.	483	0·25	Died in 3 days.	459	0·25	Died in 40 hours.
Filtrate.....	435	1·00	No reaction.	490	1·00	No reaction.	413	0·25	Died in 3 days.	472	0·25	Died in 40 hours.

TABLE V.

Showing Influence of Percentage of Peptone and of Degree of Alkalinity upon the Production of Toxin.

Peptone.	C. c. of normal solution of NaOH to litre of neutral broth.	CULTURES. No. 8—First day. Weight of guinea-pig in gms.	Am't inoculated, c. c.	Result of inoculation.	No. 8—Fifth day. Weight of guinea-pig in gms.	Am't inoculated, c. c.	Result of inoculation.	R—Ninth day. Weight of guinea-pig in gms.	Am't inoculated, c. c.	Result of inoculation.	J—Seventeenth day. Weight of guinea-pig in gms.	Am't inoculated, c. c.	Result of inoculation.
Per ct.													
1	0	325	0·10	Slight induration.	253	0·10	D. in 26 h.	335	0·10	Died in 40 hours.	595	0·10	Died in 3 days.
1	5	445	0·10	" "	453	0·10	" 40 "	425	0·10	" 3 days.	503	0·10	" 4 "
1	10	440	0·10	Died in 4½ days.	435	0·10	" 40 "	412	0·10	Slight infiltration.	480	0·10	Considerable infiltration.
1	15	...	....	.....	...	...	.....	...	....	.....	307	1·00	Died in 40 hours.
1	"	340	1·00	Slight induration.	425	1·00	" 2½ d.	470	1·00	Died in 27 days.	536	0·10	No reaction.
2	0	497	0·10	No local reaction.	435	0·10	" 40 h.	370	0·10	" 2½ "	645	0·10	Died in 2½ days.
2	5	324	0·10	Died in 3 days.	430	0·10	" 26 "	351	0·10	" 40 hours.	533	0·10	Died in 40 hours.
2	10	468	0·10	No reaction.	490	0·10	" 40 "	325	0·10	" "	557	0·10	" "
2	15	...	....	.....	...	....	.....	...	....	.....	345	1·00	" 45 hours.
2	"	432	1·00	Slight induration.	590	1·00	" 3½ d.	480	1·00	No reaction.	450	0·10	No reaction.
4	0	575	0·10	" "	410	0·01	" 45 h.	465	0·10	Died in 40 hours.	485	0·10	Died in 45 hours.
		Second day.											
4	5	500	0·10	Died in 24 hours.	520	0·01	" 40 "	448	0·10	" "	555	0·10	Died in 2½ days.
		First day.											
4	10	496	0·10	Slight induration.	395	0·01	" 40 "	560	0·10	" "	498	0·10	" "
4	15	...	....	.....	484	0·01	No local reaction.						
4	"	421	1·00	Much infiltration.	298	1·00	D. in 17 h.	495	0·10	" "	550	0·10	" 3 days.

guinea-pigs in one-cubic-centimetre doses. These tests would seem to show that when under certain conditions toxin is not found in the first days in a culture, it is not because it is locked up in the bodies of the bacilli, but that the conditions are such that the bacilli, although growing perhaps vigorously and possessing full virulence, do not produce toxin.

Influence of Percentage of Peptone and Degree of Alkalinity upon the Production of Toxin.

We notice in the experiments of Table V that culture 8, which gave the strongest toxin, produced it most quickly with the ten-cubic-centimetre alkalinity in one per cent peptone and with the five-cubic-centimetre alkalinity in the two-per-cent solution. After the third day the toxin was equally strong in the neutral, five- and ten-cubic-centimetre alkalinities for both percentages of peptone. The four-per cent peptone gave nearly the same results, except that the toxin was somewhat stronger.

With all three amounts of peptone fifteen cubic centimetres of normal soda solution to the litre greatly lessened the formation of toxin. In the earlier experiments still larger amounts of alkali—twenty to thirty cubic centimetres to the litre—almost completely prevented the development of toxin, though not materially inhibiting the growth of the bacilli after the first few days.

In culture R the results were similar for one- and two-per-cent cent peptone bouillon, but with four per cent peptone about equal amounts of toxin were produced in all four degrees of alkalinity. With culture J toxin was produced under the same conditions as in R, except that the first toxin produced in the broth with one per cent peptone was in that containing fifteen cubic centimetres of alkali to the litre.

These experiments indicate, therefore, that the diphtheria bacilli will produce strong toxin when the quantities of alkali and of peptone vary within considerable limits. With neutral broth toxin is usually produced, but not so surely, and, as a rule, not so quickly nor to such an extent as in that to which five to seven cubic centimetres

of alkali have been added per litre. The added alkali is probably necessary in bouillon containing considerable traces of glucose. With ten cubic centimetres to the litre the production of toxin is a little less reliable, and with fifteen cubic centimetres much less so. With four per cent peptone we have found the toxin to be more constantly produced in the decidedly alkaline bouillon, with all the degrees of alkalinity employed.

For the production of toxin on a large scale we have usually adopted the custom of dividing the bouillon into several portions. To one of these two per cent peptone is added, and to the others one per cent and four per cent. To the whole after neutralizing to litmus we add five to ten cubic centimetres of normal soda solution to the litre. The larger amount of alkali is added to the bouillon containing four per cent peptone. From repeated trials we have found the two-per-cent and four-per-cent peptone bouillon to have averaged a stronger toxin than the one-per-cent, but not infrequently in single trials the one per cent peptone has given the strongest toxin. Larger amounts of peptone (six per cent to ten per cent) have been tried once experimentally. In this single trial the toxin in the cultures on the seventh day was somewhat stronger in the bouillon containing the larger amounts of peptone, as is shown in the following table:

TABLE VI.

Showing Relation of Toxicity to Percentage of Peptone.

Amount of Peptone added.	Toxicity.		
	Weight of guinea-pig in gms.	Amount inoculated in c. c.	Result of inoculation.
Per cent.			
2	298	0·01	Died in 3 days.
4	355	0·01	" 40 hours.
6	562	0·01	" 3 days.
8	745	0·01	" 3½ "
10	675	0·01	" 40 hours.

The neutralization of the bouillon in these experiments was done by Mr. James A. Miller, assistant chemist to the laboratory. In doing this it developed that the amount of peptone added to broth influences greatly its reaction to phenolphthalein; also with different

peptones, even from the same house, the reactions to this indicator vary considerably. The peptone which we use requires in a two-per cent solution ten cubic centimetres of normal NaOH to the litre to neutralize to phenolphthalein. In a four-per-cent solution twenty cubic centimetres to the litre are required. As peptone is decidedly alkaline to litmus, it will be seen that the greater the amount added, the greater the alkalinity of the broth to this indicator, while, on the other hand, to phenolphthalein the acidity is increased at the rate of five cubic centimetres normal solution to the litre for every one per cent added. We have found for diphtheria cultures the litmus to be the best indicator if different percentages of peptones are employed. It is perfectly possible, however, after having decided the proper degree of alkalinity for each percentage of each variety of peptone, to use phenolphthalein for future testing.

The Relation between the Reaction of the Broth Cultures and the Amount of Toxin.

Litmus paper was the indicator used in determining the reaction. The guinea-pigs into which the inoculations were made weighed from four to five hundred grammes. The amount inoculated in each instance, except those indicated, was 0·1 cubic centimetre.

The results from these experiments are given in Table VII, on the following page. The broth, after becoming alkaline in any culture, continued so as long as tested. These results show that strong toxin is produced under certain conditions while the broth is still acid. This occurred in those cases where the period of acid reaction was brief and where the amount of acid was not very great. In those cases where the period of acid reaction is longer little if any toxin seems to be produced until just before the change to alkalinity. The amount of acid present in the broth, therefore, must be small to allow the development of toxin.

TABLE VII.

Showing the Relation between the Reaction of the Broth Cultures and the Amount of Toxin.

CULTURES.	1ST DAY.		2D DAY.		3D DAY.		4TH DAY.		5TH DAY.		12TH DAY.		18TH DAY.		35TH DAY.	
	Reaction.	Toxicity.	Reaction.	Toxicity	Reaction.	Toxicity.	Reaction.	Toxicity.	Reaction.	Toxicity.	Reaction.	Toxicity.	Reaction.	Toxicity.	Reaction.	Toxicity.
8—1 per cent peptone, 0 c. c. alkali.	Acid.	Slight infiltration.	Acid.	Death in 4½ days.	Neutral.	Death in 40 hrs.	Alk.	.....	Alk.	Death in 26 hrs.	Alk.	.....	Alk.	.....	Alk.	
8—1 per cent peptone, 5 c. c. alkali.	Acid.	Slight infiltration.	Acid.	Death in 24 hrs.	Alk.	Death in 23 hrs.	Alk.	.....	Alk.	Death in 40 hrs.	Alk.	.....	Alk.	.....	Alk.	
8—2 per cent peptone, 0 c. c. alkali.	Acid.	(1·0 c. c.) slight infiltration.	Acid.	(1·0 c. c.) death in 5½ days.	Acid.	(1·0 c. c.) death in 40 hrs; no local reaction.	Acid.	Death in 2½ days.	Alk.	Death in 40 hrs.	Alk.	.....	Alk.	.....	Alk.	
8—2 per cent peptone, 5 c. c. alkali.	Acid.	Death in 3 days.	Acid.	Death in 40 hrs.	Alk.	Death in 40 hrs.	Alk.	.....	Alk.	Death in 40 hrs.	Alk.	.....	Alk.	.....	Alk.	
R—1 per cent peptone, 0 c. c. alkali.	Acid.	(1·0 c. c.) no local reaction.	Acid.	(1·0 c. c.) no local reaction.	Neutral.	(¼ c. c.) death in 2½ days.	Alk.	(¼ c. c.) death in 3 days.	Alk.	Death in 2½ days.	Alk.	.....	Alk.	.....	Alk.	
8^3—2 per cent peptone, 5 c. c. alkali.	Acid.	.....	Acid.	.....	Acid.	.....	Acid.	.....	Acid.	.....	Acid.	(½ c. c.) no local reaction.	Alk.	Death in 18 hrs.	Alk.	
8^4—1 per cent peptone, 5 c. c. alkali.	Acid.	.....	Acid.	.....	Acid.	.....	Acid.	.....	Acid.	.....	Acid.	(1 c. c.) no local reaction.	Acid.	.....	Acid.	(1 c. c.) no local reaction.

The Influence of Glucose upon the Growth of the Diphtheria Bacilli and upon the Production of Toxin.

Buchner * and Behring † mention that bacteria produce acid in media containing sugar. Theobald Smith ‡ made a more thorough investigation of this subject. He examined bouillon made from forty-four samples of meat. Of these, eleven contained no glucose, so far as indicated by the fermentation test. The remaining seventy-five per cent contained from faint traces up to as much as 0·3 per cent of glucose. In bouillon in which no sugar, or at most only a trace of sugar, was present no appreciable acid was produced by the growth of bacteria. He found that as much as 0·3 per cent of glucose favoured the growth of many bacteria, while over five per cent was, as a rule, harmful. He noted that when the growth of the bacteria continued abundant the acid reaction changed to the alkaline within a short time, but that where the amount of acid produced was sufficient to inhibit the growth of the bacteria, alkali was no longer produced, and the culture bouillon remained permanently acid. He considers that small amounts of glucose may be a valuable nutrient to the bacteria, or, on the other hand, that the small amount of acid produced may be of advantage in preventing a too intense alkalinity.

The especial influence of the presence of glucose in the broth upon the growth of the diphtheria bacillus and upon the production of toxin has been lately investigated by Spronck # and van Furenhout. They found that if to a bouillon which contained no glucose and in which the growth of the diphtheria bacilli caused no acid there was added 0·15 per cent of glucose, the character of the growth changed, as shown in the following summary:

1. Bouillon containing no glucose. The bacilli develop rapidly; the broth is turbid and remains alkaline. Type B of Spronck.

2. Bouillon type B to which 0·15 per cent glucose is added.

* Buchner. *Arch. f. Hygiene*, Bd. iii, p. 361, 1885.
† Behring. *Zeitschr. f. Hygiene*, Bd. vii, p. 178, 1889.
‡ Theobald Smith. *Centralblatt für Bakteriologie und Parasitenk.*, Bd. xviii, No. 1, 1895.
# Spronck. *Annales de l'Institut Pasteur*, October 25, 1895.

Growth at first active. Reaction of bouillon changes to acid, and growth of bacilli decreases. After ten days to two weeks the reaction becomes alkaline and the bouillon turbid, the growth of the bacilli increasing. Type C.

3. Bouillon type B to which 0·2 per cent glucose is added. The bouillon soon becomes acid, and this reaction is usually permanent. The growth of the bacilli is scanty. Type A.

4. Bouillon type B to which glucose is added in the ratio of 1 to 1,000. The bouillon remains alkaline, the alkalinity being at first slightly reduced and, after the fifth day, greater than at first.

Type B gives the greatest amount of toxin, type C the next, and type A the least. In type A there may be none whatever produced. Spronck states that meat, as a rule, contains sufficient glucose to give a growth of type A. That if such meat is kept for a number of days until it becomes slightly putrid, the glucose will be destroyed by fermentation, and that the growth of the bacilli in the bouillon from such meat will be of the type B.

Original investigations. It is probable that the conditions under which cattle live and the different laws affecting their slaughter and the sale of their meat for food may have an influence upon the amount of glucose present in the beef of different countries.

In New York it is practically impossible to obtain the absolutely fresh meat spoken of by Spronck, since the law prevents the sale of meat for food until forty-eight hours after the slaughter. Indeed, meat as ordinarily obtained at the retail butchers has been kept from five to seven days. Bouillon from such meat has proved, almost without exception, suitable for the growth of the diphtheria bacilli and for the production of strong toxin. As tested chemically, these lots of meat have contained but small traces of glucose.

The usual type of growth has been one active from the start, the broth having an acid reaction only upon the second and perhaps third day.

The development of the toxin has been similar to that in the two experiments previously described. In cultures from only two out of

fourteen lots of bouillon was the acid stage protracted. In one the bouillon remained acid until the fourteenth day, and then became decidedly alkaline (Spronck's type C). In the other the culture remained permanently acid in the one-per-cent peptone bouillon without the production of toxin (Spronck's type A). In two-per-cent peptone bouillon, however, the growth in the latter was of type C.

In order to carry out the plan suggested by Spronck we sent directly to the slaughterhouse and obtained a large quantity of chopped beef from a recently killed animal. A portion of this meat was immediately boiled in the usual amount of water, another portion was soaked over night, another for five and a half days, and the remainder left soaking for two days longer until it became slightly putrid. The bouillon made from the fresh meat was less acid than that from the kept meat. That soaked five days required the addition of three cubic centimetres, and that seven and a half days nine cubic centimetres of normal soda solution to each litre to make them of the same reaction as the bouillon from the fresh meat. The bouillon in all four lots after being neutralized to litmus had seven cubic centimetres of normal soda solution added to each litre. In both the four-per-cent peptone and the two-per-cent peptone bouillon made from all four lots the diphtheria bacillus grew equally well. The period of acid reaction was very brief in all, but was most marked in the bouillon from the meat kept five days. To a portion of each of the four lots an extra twelve cubic centimetres of alkali was added. In these there was no stage of acid reaction whatever.

We have not found, therefore, that the amount of glucose present in the meat obtained by us in the usual way is apt to be of any serious hindrance to the abundant and rapid production of toxin. By this we do not mean to indicate that in meat obtained from other sources it might not be in sufficient amounts to be deleterious.

To test the effects of different amounts of glucose in broth we added from 0·05 to six per cent to different lots. To one lot of neutral bouillon in which the growth of the bacilli produced no acid the

addition of 0·1 per cent of glucose produced no appreciable effect; 0·2 per cent of glucose, however, entirely changed the character of the growth. The bouillon became acid on the first day and remained so. The growth of the bacilli was stopped and no toxin was produced. In another sample of bouillon, however, which contained four per cent peptone and was strongly alkaline to litmus (thirteen cubic centimetres to the litre), and gave to phenolphthalein a reaction of 0·25, an amount of glucose up to 0·5 per cent had no inhibiting effect on the growth of the bacillus, nor on the production of toxin, 0·01 cubic centimetre of a five days' filtrate killing a four hundred gramme guinea-pig in three days. The acid reaction developed at the end of twenty-four hours. At the end of forty-eight hours the bouillon was again alkaline. One per cent glucose added to this bouillon was sufficient to cause such a production of acid that the further growth of the bacilli was prevented. Indeed, on the seventh day the culture was sterile.

A curious result appeared when the acidity of the cultures to which different amounts of glucose had been added was tested with phenolphthalein. All the cultures in the same bouillon to which different amounts of glucose had been added and in which the acid reaction remained permanent were found not only intensely acid, but also equally acid. This seemed to us to indicate that the bacilli had grown in all the samples of bouillon containing the different amounts of glucose until the acidity had become so excessive that their further growth was prevented. Those cultures in which the glucose was entirely consumed before the growth of the bacilli was stopped became alkaline as the development of the bacilli continued. We found that cultures in which the acid produced was great enough to stop the growth of the bacilli again developed if sufficient alkali was added. In the bouillon containing excessive amounts of glucose, with each new addition of alkali there would be a new growth of bacilli and a new production of acid, until finally, all the glucose having been used up, the alkaline reaction became permanent and the culture became that of type C. These incomplete experiments indicate that the composi-

tion of the bouillon, and especially its degree of alkalinity, influence greatly the effect of whatever amounts of glucose may be present in the meat.

Summary.

Toxin of sufficient strength to kill a 400-gramme guinea-pig in three days and a half in a dose of 0·025 cubic centimetre developed in suitable bouillon, contained in ordinary Erlenmeyer flasks, within a period of twenty-four hours. In such bouillon the toxin reached its greatest strength in from four to seven days (0·005 cubic centimetre killing a 500-gramme guinea-pig in three days). This period of time covered that of the greatest growth of the bacilli, as shown both by the appearance of the culture and by the number of colonies developing on agar plates.

The bodies of the diphtheria bacilli did not at any time contain toxin in considerable amounts.

The type of growth of the bacilli and the rapidity and extent of the production of toxin depended more on the reaction of the bouillon than upon any other single factor.

The best results were obtained in bouillon which, after being neutralized to litmus, had about seven cubic centimetres of normal soda solution added to each litre. An excessive amount of either acid or alkali prevented the development of toxin.

Strong toxin was produced in bouillon containing peptone ranging from one to ten per cent. The strength of toxin averaged greater in the two- and four-per-cent peptone solutions than in the one-per-cent.

When the stage of acid reaction was brief and the degree of acidity probably slight, strong toxin developed while the culture bouillon was still acid; but when the stage of acid reaction was prolonged, little if any toxin was produced until just before the fluid became alkaline.

Glucose is deleterious to the growth of the diphtheria bacillus and to the production of toxin when it is present in sufficient amounts to cause by its disintegration too great a degree of acidity

in the fluid culture. When the acid resulting from decomposition of glucose is neutralized by the addition of alkali the diphtheria bacillus again grows abundantly. Glucose is not present, at least as a rule, in sufficient amounts in the meat as obtained from the New York butchers to prevent the rapid production of strong toxin if the bouillon is made sufficiently alkaline.

In our experiments, when other conditions were similar, the strength of the toxin was in proportion to the virulence and vigour of growth of the bacillus employed.

THE MUCIN OF WHITE FIBROUS CONNECTIVE TISSUE.

By R. H. CHITTENDEN, Ph. D.,
AND
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(*Contributions from the Sheffield Biological Laboratory of Yale University.*)

All of the bodies belonging to the group of mucins and mucoids are possessed of considerable physiological interest, owing especially to their peculiar compound nature and the illustration which they afford of a possible intimate union between the proteid group and carbohydrate radicles. That there are a number, possibly a large number, of closely related bodies belonging to the mucins and mucoids there can be no question. Thanks to the labours of Hammarsten * and his pupils, many of these bodies have been subjected to careful and thorough investigation, and much light has been thrown upon their relationships and differences. There is still, however, much to be ascertained regarding these bodies, and any additional facts broadening or substantiating our present knowledge are to be welcomed as contributing toward a more complete understanding of their genetic relationships. The union of carbohydrate groups with proteid molecules is probably more common than has hitherto been supposed, as witness the peculiar gluco-nucleoproteid recently described by Hammarsten † as a constituent of the pancreas and other glands, and the identification by Kossel ‡ of a peculiar carbohydrate group as a cleavage product of certain forms of nucleic acid. Presumably in these compound proteids of the mucin type the character of the proteid radicle as well as of the carbohydrate radicle is subject to variation, and it is easy to conceive of differences in the nature and properties of the mucins dependent upon varia-

* Pflüger's *Archiv f. Physiol.*, Band xxxvi; *Zeitschr. f. physiol. Chem.*, Band x and xii.
† *Zeitschr. f. physiol. Chem.*, Band xix.
‡ Du Bois-Reymond's *Archiv f. Physiol.*, Physiol. Abtheil., 1891.

tions in the amount and character of both the carbohydrate and proteid groups. The ready formation of acid-albumin, or syntonin, albumoses and peptone when mucins are decomposed by the action of superheated water or boiling dilute acids, affords ample evidence of the presence of true proteid radicles in the bodies of this class, although we do not know definitely the exact nature of the proteid groups present in the original molecule. On the other hand, the simultaneous formation of reducing bodies whenever mucins are broken down by the action of dilute acids, and the separation of a dextrin-like body (the animal gum of Landwehr*) by cleavage with superheated water, clearly indicate the existence of some form of carbohydrate matter in the mucin molecule.

Of the true mucins present in the tissues of the higher animals, the mucin of the submaxillary gland and the corresponding body present in or between the fibres of ordinary connective tissue are the most important from a physiological standpoint. The former is a product of the metabolic activity of secretory cells which are among the most active of the secreting cells of the body, while the latter is a product of a tissue whose activity is certainly of a low order. That these two mucins, though closely related, are unlike, is clearly indicated by their divergence in chemical composition as well as by their general reactions and properties.

Loebisch,† whose careful study of the mucin from tendons constitutes the chief source of our knowledge regarding the chemical composition of this body, ascribes to tendon-mucin the formula $C_{160}H_{256}N_{32}S_1O_{80}$, with a molecular weight of 3,936. Such a formula calls for the presence of 0·81 per cent of sulphur, and this amount was found by Loebisch in the three preparations of mucin from ox tendons analyzed by him. In a recent examination of mucin prepared from this same source we have obtained quite different results as regards the content of sulphur, and this fact has led us to make a careful study of the composition of this form of connective-tissue

* *Zeitschr. f. physiol. Chem.*, Band viii and ix. Also Pflüger's *Archiv f. Physiol.*, Band xxxix and xl.

† *Zeitschr. f. physiol. Chem.*, Band x, p. 40.

mucin. Our results in the main have afforded a close substantiation of the conclusions arrived at by Loebisch, with the single exception of the sulphur for which we can find no adequate explanation. Further, some additional facts have been found which are perhaps worthy of note.

The first sample of mucin studied was prepared from the Achilles tendons of oxen by the following method, analogous to the method described by Loebisch: The fresh tendons were freed as carefully as possible from all adherent tissues, then cut into very thin transverse sections with a razor, washed thoroughly with distilled water, frequently renewed for twenty-four hours, in order to remove all blood and soluble albuminous matter, and finally pressed as dry as possible. The resultant material weighed 1,200 grammes. In order to extract the mucin, the tissue was placed in 2·4 litres of half-saturated lime-water, where it was allowed to remain for forty-eight hours with frequent agitation. At the end of this period the pale-yellowish fluid was strained through a cloth filter and finally filtered through paper. The clear fluid was then treated with an excess of 0·2-per-cent hydrochloric acid—a little more than a litre—by which a heavy flocculent precipitate resulted, quickly settling to the bottom of the cylinder, leaving a nearly clear supernatant fluid.

The residue of tendon-tissue was again extracted for forty-eight hours with 2·4 litres of half-saturated limewater, and the resultant solution precipitated with an excess of 0·2-per-cent hydrochloric acid. The precipitate so formed was nearly as heavy as the first, thus showing that extraction of the mucin by weak limewater is a slow and gradual process.

The precipitated mucin, separated from the acid fluid by subsidence and decantation of the supernatant liquid, was washed thoroughly with 0·2-per-cent hydrochloric acid, whipping up the precipitate with the fluid and then allowing it to subside, this operation being repeated with fresh quantities of acid until the latter failed to give any proteid reaction. In this manner it was hoped to remove all adherent albuminous matter extracted from the tissue by the limewater. The two portions of mucin were then united and washed

by decantation with distilled water until the acid was entirely removed. As the fluid became less and less acid, more time was required for the precipitate to settle, as the latter tended to swell in the water and was more inclined to float on the surface of the fluid.

The mucin was next dissolved in half-saturated limewater, of which a large volume was required, the solution filtered through paper, and the mucin reprecipitated by the addition of an excess of 0·2-per-cent hydrochloric acid, a small quantity of stronger hydrochloric acid being likewise added to induce a good flocculent separation of the substance. The precipitate was again washed by decantation with 0·2-per-cent hydrochloric acid, and lastly with water, until the acid was entirely removed. Whenever it was necessary for the precipitate to stand for some time with water, the mixture was kept as cool as possible, and a little alcoholic solution of thymol added to guard against putrefactive changes. When the acid was wholly removed from the precipitate the water was replaced by weak alcohol, and finally by ninety-five-per-cent alcohol, repeatedly renewed, until the substance was thoroughly dehydrated, after which the precipitate was collected on a filter and allowed to drain. It was then boiled with alcohol-ether (a mixture of equal parts absolute alcohol and ether) in a suitable flask connected with an inverted Liebig's condenser for many days—i. e., with renewed quantities of alcohol-ether until the latter gave no residue on evaporation. As Loebisch has shown, this is quite an important part of the process of purification, since a certain amount of foreign extractive matter adheres tenaciously to the mucin, and can be removed only by long-continued extraction with the above mixture. When this process was completed the mucin was thrown upon a filter, washed thoroughly with ether, and finally dried over sulphuric acid. When quite dry it presented the appearance of a perfectly white powder, light and fluffy. The yield amounted to twelve grammes of the dry product, and, assuming that the entire amount of mucin had been extracted from the tendons, and disregarding the loss incidental to purification, this quantity would imply the presence in the fresh tendons of one per cent of mucin.

The composition of the product, dried at 110° C. until of constant weight, was as follows:*

PREPARATION No. 1.

I. 0·2670 gramme of substance gave 0·4781 gramme of $CO_2 = 48·84$ per cent C, and 0·1585 gramme of $H_2O = 6·60$ per cent H.

II. 0·2277 gramme of substance gave 0·4082 gramme of $CO_2 = 48·89$ per cent C, and 0·1329 gramme of $H_2O = 6·48$ per cent H.

III. 0·1975 gramme of substance gave 0·3548 gramme of $CO_2 = 48·99$ per cent C.

IV. 0·2363 gramme of substance gave 0·1417 gramme of $H_2O = 6·66$ per cent H.

V. 0·2426 gramme of substance gave, by the Kjeldahl method, 0·02865 gramme of nitrogen = 11·81 per cent N.

VI. 0·2754 gramme of substance gave, by the Kjeldahl method, 0·03246 gramme of nitrogen = 11·79 per cent N.

VII. 0·2784 gramme of substance gave, by the absolute method, 27·63 c. c. of nitrogen at 13·3° C, and 764·7 mm. pressure = 11·96 per cent N.

VIII. 0·3345 gramme of substance gave, by the absolute method, 33·3 c. c. of nitrogen at 13·2° C, and 754·5 mm. pressure = 11·84 per cent N.

IX. 0·5373 gramme of substance gave, by fusion with $NaOH + KNO_3$, 0·0943 gramme of $BaSO_4 = 2·41$ per cent S; after deducting sulphur of ash = 2·36 per cent S.

X. 0·4969 gramme of substance gave, by fusion with $NaOH + KNO_3$, 0·0856 gramme of $BaSO_4 = 2·37$ per cent S; after deducting sulphur of ash = 2·32 per cent S.

XI. 0·2943 gramme of substance gave 0·0023 gramme of ash = 0·78 per cent ash.

XII. Ash from 0·2943 gramme of substance gave 0·00112 gramme of $BaSO_4 = 0·05$ per cent S.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

											Average.
C.	49·22	49·27	49·37	....	.....	.....	.....	.....	....	....	49·29
H.	6·65	6·54	.....	6·71	.....	.....	.....	.....	....	....	6·63
N.	.....	.....	.....	....	11·90	11·88	12·05	11·93	....	....	11·94
S.	.....	.....	.....	....	.....	.....	.....	.....	2·36	2·32	2·34
O.	.....	.....	.....	....	.....	.....	.....	.....	....	....	29·80
											100·00

The second preparation of mucin was made in a somewhat different manner. The fresh tendons, freed as far as possible from foreign tissue, were cut into thin transverse sections, washed with water somewhat, then soaked for thirty-six hours in about four litres of ten-per-cent salt solution, with vigorous agitation from time to time, after which the saline solution was decanted and the tissue washed with water until the chloride was entirely removed. The

* The nitrogen was determined by both the absolute and the Kjeldahl method, while carbon and hydrogen were determined by combustion in oxygen gas in an open tube, the products of combustion passing over a layer of cupric oxide, chromate of lead, and metallic copper.

salt solution on dilution with water gave a distinct turbidity indicating the presence of a globulin. The application of heat likewise produced a precipitate, as did also the addition of dilute acetic and hydrochloric acids. It is thus evident that the salt solution removes at the outset quite an appreciable amount of proteid matter, with perhaps some mucin. The moist tissue, pressed as dry as possible, weighed 1,700 grammes. It was then extracted with 3·4 litres of half-saturated limewater for forty-eight hours, two such extractions being made. From these extracts the mucin was precipitated by the addition of 0·2-per-cent hydrochloric acid, the second extract apparently yielding as heavy a precipitate as the first. The combined precipitates were washed repeatedly by decantation with 0·2-per-cent hydrochloric acid, lastly with water. The mucin was next dissolved in a little 0·5-per-cent sodium carbonate, the solution filtered, made nearly neutral by the addition of a little ten-per-cent hydrochloric acid, so as to avoid undue dilution, and then precipitated by 0·2-per-cent hydrochloric acid. The precipitate was again washed thoroughly with 0·2-per-cent hydrochloric acid, and lastly with water, until the acid was entirely removed. It was then transferred to ninety-five-per-cent alcohol, frequently renewed, and finally boiled with alcohol-ether as long as anything could be extracted. Dried over sulphuric acid, the product came out quite white, but not so bulky as the preceding preparation, and weighed a little over fifteen grammes—an amount equal to about 0·9 per cent of the moist tissue.

As already stated, mucin is not readily extracted from tendons by limewater; at least four cubic centimetres of half-saturated limewater are required for every gramme of tissue in order to insure a complete extraction. Thus, after the second extraction of the above 1,700 grammes of tissue, a third extraction was made, using again three litres of half-saturated limewater. This solution, on treatment with hydrochloric acid, gave a precipitate weighing one to two grammes when purified, but it was noticeable that more acid was required in order to effect a good flocculent separation of the mucin. Even with a fourth extraction of the tissue a little mucin was obtained, showing as a decided turbidity when the alkaline fluid was

made distinctly acid, but it was not until four or five days' standing that a distinct precipitate settled out even on the addition of stronger hydrochloric acid. The amount so obtained, however, was very small.

The composition of the main product obtained from the 1,700 grammes of tissue when dried at 110° C., until of constant weight, was as follows:

PREPARATION No. 2.

I. 0·3194 gramme of substance gave 0·5659 gramme of CO_2 = 48·32 per cent C, and 0·1815 gramme of H_2O = 6·31 per cent H.

II. 0·4197 gramme of substance gave 0·7471 gramme of CO_2 = 48·54 per cent C, and 0·2446 gramme of H_2O = 6·47 per cent H.

III. 0·4051 gramme of substance gave 0·7189 gramme of CO_2 = 48·39 per cent C, and 0·2353 gramme of H_2O = 6·45 per cent H.

IV. 0·2519 gramme of substance gave, by the Kjeldahl method, 0·02965 gramme of nitrogen = 11·77 per cent N.

V. 0·2578 gramme of substance gave, by the Kjeldahl method, 0·03026 gramme of nitrogen = 11·74 per cent N.

VI. 0·2954 gramme of substance gave, by the Kjeldahl method, 0·03446 gramme of nitrogen = 11·67 per cent N.

VII. 0·6610 gramme of substance gave, by fusion with $NaOH + KNO_3$, 0·1131 gramme of $BaSO_4$ = 2·35 per cent S; after deducting sulphur of ash = 2·32 per cent S.

VIII. 0·5248 gramme of substance gave, by fusion with $NaOH + KNO_3$, 0·0936 gramme of $BaSO_4$ = 2·45 per cent S; after deducting sulphur of ash = 2·42 per cent S.

IX. 0·6724 gramme of substance gave, by fusion with $NaOH + KNO_3$, 0·1140 gramme of $BaSO_4$ = 2·33 per cent S; after deducting sulphur of ash = 2·30 per cent S.

X. 0·3735 gramme of substance gave 0·0025 gramme of ash = 0·67 per cent ash.

XI. Ash from 0·3735 gramme of substance gave 0·00082 gramme of $BaSO_4$ = 0·03 per cent S.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

										Average.
C.	48·64	48·87	48·72	.....	.....	.....	....	....	....	48·74
H.	6·36	6·52	6·50	.....	.....	.....	....	....	....	6·46
N.	.....	.....	.....	11·85	11·82	11·74	....	....	....	11·80
S.	.....	.....	.....	.....	.....	.....	2·32	2·42	2·30	2·35
O.	.....	.....	.....	.....	.....	.....	....	....	....	30·65
										100·00

A third specimen of mucin was prepared as follows: Fifteen hundred grammes of ox tendons were finely divided, the tissue extracted for twenty-four hours with three litres of ten-per-cent salt solution, and then with water until the salt was wholly removed. The tissue was next extracted for sixty hours with three litres of half-saturated limewater. From this solution the mucin could be only partially

separated by the addition of 0·2-per-cent hydrochloric acid, quite a quantity of ten-per-cent acid being required to effect a flocculent precipitation of the substance. This was purified by itself and not subjected to analysis. The tendons were again extracted with three litres of half-saturated limewater for forty-eight hours, and from this solution the mucin was separated as a flocculent precipitate by the addition of 0·2-per-cent hydrochloric acid. This precipitate was purified by washing with 0·2-per-cent hydrochloric-acid, solution in 0·5-per-cent sodium carbonate, reprecipitation with 0·2-per-cent hydrochloric acid, etc., as described under the last preparation. The yield of dry product from this second extraction of the tissue with limewater amounted to 6·5 grammes. Dried at 110° C. until of constant weight, this preparation gave the following results on analysis:

Preparation No. 3.

I. 0·3598 gramme of substance gave 0·6292 gramme of $CO_2 = 47·69$ per cent C, and 0·2072 gramme of $H_2O = 6·40$ per cent H.

II. 0·2939 gramme of substance gave 0·5150 gramme of $CO_2 = 47·79$ per cent C, and 0·1725 gramme $H_2O = 6·52$ per cent H.

III. 0·3154 gramme of substance gave 0·5536 gramme of $CO_2 = 47·87$ per cent C.

IV. 0·1644 gramme of substance gave 0·0944 gramme of $H_2O = 6·38$ per cent H.

V. 0·1965 gramme of substance gave, by the Kjeldahl method, 0·02255 gramme of nitrogen = 11·47 per cent N.

VI. 0·2495 gramme of substance gave, by the Kjeldahl method, 0·02825 gramme of nitrogen = 11·32 per cent N.

VII. 0·2574 gramme of substance gave, by the Kjeldahl method, 0.02930 gramme of nitrogen = 11·38 per cent N.

VIII. 0·6046 gramme of substance gave, by fusion with $NaOH + KNO_3$, 0·1045 gramme of $BaSO_4 = 2·38$ per cent S; after deducting sulphur of ash = 2·31 per cent S.

IX. 0·5408 gramme of substance gave, by fusion with $NaOH + KNO_3$, 0·0931 gramme of $BaSO_4 = 2·37$ per cent S; after deducting sulphur of ash = 2·30 per cent S.

X. 0·3128 gramme of substance gave 0·0031 gramme of ash = 0·99 per cent ash.

XI. Ash from 0·3128 gramme of substance gave 0·00152 gramme of $BaSO_4 = 0·07$ per cent S.

Percentage Composition of the Ash-free Substance.

										Average.
C.	48·17	48·26	48·34	....	.....	.....	.....	....	....	48·26
H.	6·46	6·59	.....	6·44	.....	.....	.....	....	....	6·49
N.	.....	.....	.....	....	11·59	11·43	11·50	....	....	11·51
S.	.....	.....	.....	....	.....	.....	.....	2·31	2·30	2·31
O.	.....	.....	.....	....	.....	.....	.....	....	....	31·43
										100·00

A comparison of the composition of these three preparations of

mucin with each other, and with the mucin analyzed by Loebisch and by Hammarsten, brings out certain points of interest which merit attention:

	MUCIN FROM TENDONS.				Snail mucin. Hammarsten.	Submaxillary mucin. Hammarsten.
	Preparation 1.	Preparation 2.	Preparation 3.	Loebisch's average.		
C.....	49·29	48·74	48·26	48·30	50·32	48·84
H.....	6·63	6·46	6·49	6·44	6·84	6·80
N.....	11·94	11·80	11·51	11·75	13·65	12·32
S.....	2·34	2·35	2·31	0·81	1·75	0·84
O.....	29·80	30·65	31·43	32·70	27·44	31·20

Loebisch analyzed three distinct preparations of mucin from ox tendons, in which the carbon, hydrogen, and sulphur showed practically no variation. The nitrogen, however, varied from 11·59 to 11·84 per cent. The average content of nitrogen in his three preparations was 11·75 per cent, identical with the average of our three preparations. It is to be noticed, however, that the carbon of our preparations shows decided variation, and it is also to be observed that a diminution in the percentage of carbon is attended in each case with a diminution in nitrogen. We may suppose that Preparation No. 3 is the purest of our products, and it is seen to agree most closely with the results obtained by Loebisch, except in the content of sulphur. The mucin from the submaxillary gland, as well as the snail mucin, are both characterized by a comparatively high content of nitrogen, while the latter product also shows a higher percentage of carbon.

Our results seemingly justify the assumption that white fibrous connective tissue contains more than one mucin, or else that the mucin obtainable from this tissue is prone to carry with it a certain amount of some other form of proteid matter which the ordinary methods of purification are not wholly adequate to remove. Our experience leads us to the belief that the surest way of obtaining a pure mucin from tendons, or at least one with a low content of carbon and nitrogen, is to first extract the finely divided tissue with ten-per-cent salt solution, then after removal of the salt with water to extract the tissue with half-saturated limewater in the proportion of

two cubic centimetres for every gramme of moist tissue for about twenty-four hours at ordinary room temperature. This extract may be rejected, as it is very liable to yield a mucin with a higher content of nitrogen and carbon. By extracting the tissue a second time with limewater a mucin may be obtained with a lower content of carbon and nitrogen, as in our third preparation. It is purely an assumption, however, to say that this body with its lower percentage of carbon and nitrogen is *pure* mucin. There is at the present time no standard of purity with regard to this body, and it is quite as probable that fibrous connective tissue contains two or more mucins as that there is only one mucin in the tissue, and that any deviation from the figures obtained by Loebisch or by us in Preparation No. 3 is due to the presence of a larger or smaller amount of proteid impurity.

Undoubtedly, preliminary extraction of the tissue with salt solution tends to remove a certain amount of proteid matter, especially globulins, which might otherwise render the product impure, and possibly this is in part the cause of the lower content of carbon and nitrogen in Preparation No. 2 as contrasted with Preparation No. 1. Still there is no certainty on this point, for it is to be remembered that precipitation of the mucin requires the addition of considerable hydrochloric acid beyond neutralization of the alkaline fluid, and this excess of acid would naturally exert a marked solvent action upon any albuminous matter present. That the first limewater extract is liable to yield a mucin with a higher content of both carbon and nitrogen the results fully indicate, and as a direct illustration of the difference in the content of nitrogen in mucin obtained from a first and second extract, we may instance the following experiment: Fifteen hundred grammes of tendons finely divided, as usual, were extracted with ten-per-cent salt solution for two days, then washed with water and placed in three litres of half-saturated limewater for forty-eight hours. This first extract was then strained off, and the tissue treated a second time with a like volume of half-saturated limewater, thus giving a second extract. From the first extract, the mucin was precipitated by hydrochloric acid slightly above 0·2 per cent, the

precipitate washed with 0·2-per-cent hydrochloric acid, then with water, and lastly dissolved in 0·5-per-cent sodium carbonate. From this filtered solution a portion of the mucin was precipitated by addition of 0·2-per-cent hydrochloric acid, while a second portion separated only on addition of a somewhat increased strength of acid. These two fractions were washed thoroughly with 0·2-per-cent acid, then with water, and finally boiled with alcohol-ether until quite free from soluble matter. The yield in the first fraction was 1·4 gramme, and in the second fraction 1·0 gramme. From the second limewater extract the mucin was precipitated with 0·2-per-cent hydrochloric acid, after which it was purified by washing with 0·2-per-cent acid, solution in 0·5-per-cent sodium carbonate, reprecipitation with 0·2-per-cent acid, etc. The content of nitrogen in the three products, when dried at 110° C., was as follows, calculated on the ash-free substance:

FIRST EXTRACT.		SECOND EXTRACT.
First fraction.	Second fraction.	
12·26 N.	11·91 N.	11·51 N.

It is thus seen that the first extraction with limewater furnishes a mucin with a considerably higher percentage of nitrogen than the second extract. It is equally noticeable that the mucin first precipitated—as in the first fraction of the first extract—has a higher percentage of nitrogen than the second fraction, thus indicating that the higher content of nitrogen and probably of carbon also belongs to some body more readily precipitated by acid than the mucin with 11·51 per cent of nitrogen. In view of the great care exercised in all of these preparations, and the ready solubility of ordinary forms of albuminous matter in an excess of hydrochloric acid, especially after they have once been dissolved in an alkaline fluid, we are very much inclined to believe in the existence of several related mucins as components of ordinary white fibrillar connective tissue.

Such a view presents no great difficulty. Submaxillary mucin, for example, differs from tendon mucin by only 0·5 per cent of carbon (48·84 per cent) and about 0·5 per cent of nitrogen (12·32 per cent), although it shows some other points of difference, such as a tend-

ency to undergo alteration by the action of limewater and by being soluble in 0·2-per-cent hydrochloric acid. Indeed, all of the various mucins described show minor points of difference, although agreeing in their general reactions, and it is easy to conceive of the presence of two or more closely related mucins, in tendons, with different elementary composition.

The most remarkable thing, however, connected with the mucins that we have separated from this form of fibrillar connective tissue is the amount of sulphur present in the purified products. In snail mucin, Hammarsten has shown the presence of 1·75 per cent of sulphur, but in the mucin from the submaxillary gland and in the mucin described by Loebisch as contained in tendons, the amount of sulphur has been placed at 0·84 to 0·81 per cent. In all three of our preparations, however, the sulphur present has amounted to at least 2·30 per cent, and, moreover, the agreement in the several products has been very close indeed. The greater portion of this sulphur is closely combined, a small amount only being in the form of the mercaptan group and responding to the reaction with potassium hydroxide and plumbic acetate. We present these figures with some doubt in our own minds, but, having obtained them as the result of most careful work, we see no possible explanation other than that this amount of sulphur is actually present in the mucin molecule. The determinations of sulphur were made after the usual method recommended by Hammarsten—viz., oxidizing the mucin with a mixture of ten grammes NaOH and two grammes KNO_3 in a silver crucible, etc. The sodium hydroxide employed was chemically pure, having been prepared from the metal, and, furthermore, several blank tests were made to prove the freedom of the various chemicals from sulphur. This percentage of sulphur is greater than has ever been accredited to a true mucin, although the mucin from the snail's membrane (mantle-mucin), which is somewhat related to keratin, has been found by Hammarsten to contain a fairly large amount of this element—viz., 1·79 per cent.

With regard to the *reactions* of the several products that we have studied, there is nothing special to be said. They all show the ordi-

nary reactions of mucin as described by Loebisch, and we can simply substantiate what has long been published by him upon this point.

The most characteristic feature of mucin is the peculiar cleavage it undergoes when heated with dilute hydrochloric acid, by which a substance with reducing action upon alkaline copper solution results. Albumose and peptone are likewise formed by the action of the hot acid. We have tried several preliminary experiments in this direction, the results of which may be briefly stated: 3·25 grammes of mucin of Preparation No. 2 were heated in a boiling water-bath with one hundred cubic centimetres of two-per-cent hydrochloric acid for five hours. At the end of this period the solution was of a deep-brown colour, while suspended through the fluid was a large amount of gelatinous matter more or less brown in colour. This was filtered off, washed with water, in which it was wholly insoluble, until the washings gave no proteid reaction. It was then tested with the following results: It was insoluble in dilute and stronger hydrochloric acid, but readily soluble in 0·5-per-cent sodium carbonate and in very dilute (0·5 per cent) potassium hydroxide. From the solution in sodium carbonate, it was reprecipitated by neutralization, and was then readily soluble in a slight excess of 0·2-per-cent hydrochloric acid. It gave the ordinary colour reactions characteristic of proteid matter. Warmed at 40° C. with an active gastric juice containing 0·2-per-cent hydrochloric acid, it was wholly unaffected even after twenty-four hours, but when warmed with an alkaline pancreatic juice it was readily dissolved, and almost completely converted into products soluble even on neutralization of the fluid, thus attesting its conversion into soluble albumoses and peptones. These reactions suggest that the substance in question is a form of anti-albumid.

The original acid fluid containing the soluble products formed in the cleavage of the mucin was made neutral, by which a slight neutralization precipitate resulted, evidently syntonin from the reactions tried. The neutral fluid was then concentrated to a sirup, a strong caramel-like odour being developed during the process, and while still warm the residue was treated with a large excess of

ninety-five-per-cent alcohol, by which a thick gummy mass was formed, hard and brittle on cooling. While warm, the alcoholic fluid was quite clear and yellowish-red in colour, but on cooling, a light-yellow precipitate, very small in quantity, formed, which was soluble in water, and gave a strong reducing action with Fehling's solution. It was too small in quantity, however, to study further. The gummy precipitate was washed by warming it repeatedly with fresh quantities of alcohol. It was readily soluble in water, gave more or less of a proteid reaction, and showed a fairly strong reducing action with Fehling's solution. Tested with phenylhydrazine hydrochloride, and sodium acetate, only an amorphous precipitate resulted from which a crystalline osazone could not be obtained. On boiling the gummy mass with two-per-cent hydrochloric acid, however, and then extracting the neutralized and evaporated fluid with alcohol, a very small amount of a crystalline osazone was obtained by application of the hydrazine test, apparently identical with that described further on.

The original alcoholic solution from the above gummy precipitate was evaporated to a small bulk on the water-bath, the residue taken up with fifteen cubic centimetres of water forming a clear solution. This solution showed strong reducing action with alkaline copper solution, and evidently contained the greater portion of the reducing body formed from the cleavage of the mucin. To the main bulk of this solution was added one gramme of phenylhydrazine hydrochloride and 1·5 grammes of sodium acetate, after which the mixture was heated on the water-bath for an hour and a half, the volume of the fluid being kept at fifteen to twenty cubic centimetres. While hot the fluid was perfectly clear and reddish in colour. After standing an hour in a cool place there was a marked separation of amorphous particles and oily globules, but no crystals could be detected under the microscope. After standing fifteen hours the amorphous particles were almost wholly transformed into fine crystals. These crystals were light yellow in colour, and were mostly arranged in rosettes or balls of fine yellow needles, somewhat resembling lactosazone. The oily globules were unchanged. These

crystals were purified by dissolving them in cold alcohol, followed by the addition of water, and heating the solution until the alcohol was practically all removed, when the crystals again separated out as the fluid cooled. The crystals were also insoluble in the hot *concentrated* fluid. In this way the crystals were gradually freed from the oily globules spoken of above and rendered fairly pure. Each time the crystals were filtered they were also washed with a little cold water. During the process of purification the crystals changed their appearance somewhat, tending to take on the branching form characteristic of dextrosazone. This crystalline osazone, when purified as much as possible, was readily soluble in warm water, in alcohol, ether, chloroform, and, to a certain extent, in benzol. The amount of the purified osazone was so small that the melting point alone could be determined. This was done as usual in a capillary tube. When the temperature reached 140° C. the substance commenced to darken slowly, and at 160° C. it began to melt. Further recrystallization of the osazone did not alter this melting point. In melting point, therefore, this osazone, if pure, differs widely from dextrosazone or lactosazone. In general appearance and solubility, as well as in its melting point, it appears to resemble very closely the osazone obtained by Hammarsten from the cleavage product of the peculiar nucleoproteid described by him as present in the pancreas.* Whether this body is a pentaglucose, however, we can not definitely say. We had hoped, especially in view of the strong reducing action of the above alcoholic solution, to obtain a fairly large amount of an osazone, sufficient to determine its content of carbon and nitrogen, but the yield of purified product was very small indeed.

In order to verify the above results, a second portion of mucin was decomposed with dilute acid—4·75 grammes of mucin with two hundred and fifty cubic centimetres of 2·0-per-cent hydrochloric acid—the mixture being heated directly over a lamp for about five hours. The flask was connected with an inverted Liebig's condenser to prevent concentration, and the mixture was kept in a state of gentle ebullition. In this case there was much less of the antialbumid-like

* *Zeitschr. f. physiol. Chem.*, Band xix, p. 19.

body so prominent in the first decomposition, the amount being less than one fifth that found before. The neutralization precipitate, however, was considerably larger, and albumose and peptone were both present in abundance. The caramel-like body precipitated by alcohol was naturally more abundant than in the first case, but on analysis it was found to contain a large percentage of nitrogen, so that its fancied resemblance to caramel is purely superficial. By evaporation of the alcoholic extract containing the greater portion of the reducing body a residue was obtained as before, from which a crystalline osazone was formed agreeing in all of its properties with the body previously described. The purified osazone melted at 158° to 160° C. It is thus evident that the mucin or mucins present in ox tendons yield on cleavage with dilute hydrochloric acid a carbohydrate body which forms a well-defined and crystalline osazone, although at present we can not state definitely the exact nature of this carbohydrate substance.

ON THE ACTION OF PIPERIDINE AND SOME OF ITS COMPOUNDS.

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In the course of a research undertaken for another purpose I had occasion to compare the actions of several compounds of piperidine, and, as my results differ somewhat from those of other workers in this field, I have thought it well to publish them now, as I see no prospect of prosecuting the subject further. The substances examined were piperidine, *α*-methylpiperidine, *α*-ethylpiperidine, and coniine, which Hofmann and Ladenburg have shown to be *α*-propylpiperidine. Much of my work was done in the pharmacological laboratory of Strassburg University, the rest in that of the University of Michigan.

But little work has been done, so far as I can learn, on the action of any of these bases except coniine. Buchheim * observes that the piperidine salts act on the organism in the same way as ammonia salts, while Neumann † found that he could take sixteen grains within three and a half hours without any appreciable result. Kronecker ‡ found a certain similarity between the actions of coniine and piperidine, but ascribed the action of the former to the motor-nerve ends, that of the latter to the sensory terminal organs.

According to Fliess,# the reflex irritability of frogs is reduced or removed entirely by the injection of one milligramme of piperidine. This, he asserts, is due to a paralyzing action on the sensory terminal organs. He found also the heart and respiration in frogs slowed by the same quantity. His research loses somewhat in value by the fact that he used the pure base, which is a strongly alkaline irritant,

* Virchow's *Archiv*, lvi, p. 10.
† *Inaug. Diss.*, Dorpat, 1860.
‡ *Ber. d. deutsch. chem. Gesellsch.*, 1881, p. 712.
# *Inaug. Diss.*, Berlin, 1883. Du Bois-Reymond's *Archiv*, 1883, p. 206.

although in the small quantities used by him this irritant action would scarcely be of much importance. Goldschmitt * confirms Fliess's statements, but adds that larger doses of piperidine paralyze the motor-nerve ends also, as well as acting as depressants to the central nervous system. Oechsner de Coninck and Pinet † found that the free base caused violent inflammation on being injected subcutaneously or inhaled. Guerber ‡ found that besides acting on the peripheral nerve endings, piperidine possesses the curious property of causing the formation of large numbers of vacuoles in the red blood-corpuscles of the frog.

I used piperidine prepared from Merck's piperidine by fractional distillation; *α*-methylpiperidine and *α*-ethylpiperidine were prepared by Ladenburg's method; coniine was obtained from the commercial coniine by fractional distillation. The alkaloids were all used in the form of hydrochlorates. My experiments were carried out chiefly on *Rana esculenta*, but I could detect no difference between this and the *temporaria* in their reaction to any of the four alkaloids.

Piperidine possesses only weakly toxic properties. On frogs of 30 grammes weight I found quantities of from 1 to 15 milligrammes injected into the abdominal lymph sac utterly fail to elicit any symptoms whatever. Even after 20 milligrammes frogs often presented no abnormality, though some seemed to hop rather clumsily. After the injection of 30 milligrammes in winter frogs and of 40 milligrammes in summer frogs this clumsiness in movement became very marked. The animal remains quite still unless disturbed, and the head sinks down somewhat lower than is normal. When irritated repeatedly it crawls away or makes short leaps, in which it falls very awkwardly and has great difficulty in drawing up its hind legs. Every movement is accompanied by marked tremor, especially of the head and neck. When laid on his back the frog generally makes one or two vain efforts to recover himself, but then lies quite still.

* *Inaug. Diss.*, Wurzburg, 1884.

† *Comptes rendus de la soc. de biol.*, 1885, p. 613; 1886, pp. 217 and 461.

‡ *Arch. f. Anat. und Phys.*, 1890.

Somewhat later he can no longer hop or crawl, the respiration ceases, and pinching only causes a jerk of the foot. If the legs are stretched out they can no longer be drawn up, and he makes no attempt to turn when laid on his back. After this condition has lasted from twelve to twenty-four hours the symptoms begin to pass off, and the frog generally recovers completely in two or three days.

When 40 to 50 milligrammes are injected the same symptoms are seen, but in a still more marked form. The head now rests on the table; the muscles respond to reflex irritation only by fibrillary contraction. In some cases frogs recovered from even 50 milligrammes, but as a general rule the heart was found in diastolic standstill after a short time, and from this there was no recovery.

The muscular reaction in piperidine poisoning is a very interesting one. On direct stimulation by the interrupted current the muscle responds with an apparently normal tetanus, while on stimulation of the sciatic nerve with the same current no tetanus, but a single jerk is obtained. This reaction seemed to point to a weakening of the end plates of the motor nerves, and this was confirmed by a series of experiments in which the vessels of one leg were ligated before the poison was injected. On then stimulating the nerves at their exit from the spinal canal it was found that the muscles of the ligatured leg responded with a normal tetanus, while those of the poisoned leg gave a single jerk resembling the response to a make or break stimulus. The muscle tracing taken on a quickly rotating drum gave similar results. While the unpoisoned gastrocnemius gave the normal tetanus tracing, lasting as long as the current passed through the nerve, the poisoned muscle responded on indirect stimulation with a curve scarcely longer than that obtained from the normal muscle by a single shock. If the current was now shut off and renewed again after a short pause a second contraction could be elicited, but this was weaker and shorter than the first, and after a few repetitions no further movement of the muscle could be got from stimulation of the nerve until a long pause had intervened. On direct tetanic stimulation the muscle responded with a contraction curve which differed in no respect from the normal. To single induction shocks

applied to the nerve the muscle responded with a normal tracing. If, however, these shocks were repeated at intervals of half a second, the contractions rapidly diminished in size and disappeared entirely after ten to fifteen contractions. After no further contraction could be elicited by make-and-break stimulation of the nerve, single shocks applied to the muscle caused normal contraction, and tetanic stimulation of the nerve caused a contraction which, however, was so short as to simulate that caused by a single shock.

The explanation of the action of piperidine, then, seems to be that it renders the nerve plates in muscle more liable to fatigue. Thus, when a rapid series of impulses are sent through the nerves the first few are transmitted to the muscle, which therefore contracts in tetanus. Very soon, however, the nerve plates become incapable of transmitting impulses, and the muscle returns to rest. The nerve endings rapidly recover from the fatigue, and can again transmit a few tetanic stimuli; but as this procedure is repeated the fatigue becomes more marked, and eventually a longer period of rest is required to restore the power of transmission. When, instead of tetanic stimulation, single shocks are sent through the nerve, the same result is got, the impulses pass with increasing difficulty, and eventually fail altogether to pass through the nerve plate. The muscle contractions therefore diminish in size and eventually cease completely. At this point a series of impulses following each other in rapid succession can still pass the nerve plates and cause a contraction. This may be said to be due to a power of summation possessed by the end plates, though this is merely another way of expressing the fact.

The irritability of the nerve plate does not seem to be lessened by piperidine, for I found that the minimal current which on application to the nerve was capable of causing a muscular contraction in the normal frog continued to do so after complete poisoning by piperidine.

Even the largest doses of piperidine that could be given without causing stoppage of the heart had no further action on the nerve plates, and no complete paralysis was ever obtained in the numerous

experiments I carried out on this point. I therefore poisoned a number of frogs with quantities of curara which were too small to cause complete paralysis of the nerve plates, and obtained exactly similar results. Not only in the behaviour of the muscle on stimulation of the nerve were the results from piperidine and from small doses of curara identical, but in the general symptoms. Thus the clumsiness in leaping, the difficulty in drawing up the hind legs after a spring, and the marked tremors of the head and anterior part of the body, were all present in the curara experiments. The only difference that I could detect was in the rapidity of the onset of symptoms, as the curara frog reached the same stage in a few minutes as the piperidine frog did in several hours.

Boehm * has recently described the action of curara as causing lessened resistance to fatigue in the end plates of motor nerves, and has also noted the fact that tetanic stimulation of the nerves will cause a muscular contraction after single shocks cease to have any effect. Piperidine seems, then, to resemble curara closely in its action on the muscle plates, differing however from it in causing not complete paralysis, but marked diminution of the power of resisting fatigue. The reason why piperidine never completely paralyzes the nerve plates may be that the heart is acted on by the large quantities necessary. I have observed, however, a similar condition in experiments with gelseminine,† in which no action on the heart could be detected, and where this explanation, therefore, fails to account for the phenomenon. The tremor observed more especially in the head and anterior part of the body seems to be explicable by the animal's inability to cause tetanic contraction of the muscles when desiring to move. I have observed similar tremors in frogs poisoned by gelseminine and sparteine,‡ and probably the convulsions described as occurring in frogs after some other drugs may be due to a similar condition.

Piperidine seems to have no action on the central nervous system

* *Archiv f. exp. Path. und Pharm.*, xxxv.
† *Arch f. exp. Path. und Pharm.*, xxxi.
‡ *Archiv. f. exp. Path. und Pharm.*, xxxv, p. 129.

of the frog. In animals in which the action on the muscle plates of the hind legs was prevented by a ligature of the aorta, no convulsions were observed; and in others in which the iliac artery on one side was ligatured and the reflex irritability examined by Türck's method, no departure from the normal reflex time was observed in either leg, although the movements in the unligatured limb were reduced to weak jerks. The reflex arc is therefore unaffected except by the action on the ends of the motor nerves.

Piperidine possesses a certain weakening action on the heart when used in large quantities. Thus, when 60 milligrammes or more are injected into small frogs, the heart soon becomes slowed and weaker, and eventually ceases to beat in the diastolic position. The heart muscle has at this stage entirely lost its irritability to electric stimulation, and the action therefore seems to be a direct one on the muscle. After comparatively small doses the vagus is paralyzed, this paralysis not being preceded by any stage of stimulation so far as I could observe.

My results with piperidine, therefore, differ considerably from those obtained by earlier investigators. Thus, Fliess found that one milligramme was sufficient to cause complete disappearance of the reflexes, owing to paralysis of the sensory terminations, while I have satisfied myself that very much larger quantities are necessary to cause any symptoms in small frogs, and that none of these symptoms point to an affection of the sensory-nerve terminations. In fact, reflexes may be obtained from frogs at any time throughout the experiment. Fliess's result may possibly be due to the irritant properties of the free base he used.

I have made a number of experiments with α-methylpiperidine or pipecoline, chiefly for the purpose of determining the smallest quantity which is capable of producing symptoms in the frog. The symptoms differ from those caused by piperidine only in the fact that here complete curara paralysis can be produced without the heart's stopping. In fact, in several cases the heart continued to beat for two days after complete paralysis, and in some frogs recovery took place after this stage had been reached. In order to pro-

duce this complete paralysis 40 to 50 milligrammes of pipecoline were necessary for small frogs of 30 grammes weight.

Fliess found that after pipecoline the reflexes were first increased and then disappeared, while movements could still be elicited from stimulation of the cord and sciatic nerve. I have not observed any symptoms pointing to increased reflex irritability.

With ethylpiperidine the same symptoms were elicited, but here 15 to 20 milligrammes were found sufficient to produce complete paralysis in frogs of 30 grammes, while 10 to 12 milligrammes produced apparently the same appearances as 30 to 40 milligrammes of piperidine.

With coniine exactly the same symptoms could be elicited as with methylpiperidine and ethylpiperidine—complete paralysis, resembling that caused by curara. Somewhat smaller quantities were, however, sufficient. Thus, as a general rule, I found 10 milligrammes sufficient to completely paralyze frogs of 30 grammes weight. Coniine produces also the paralysis of the vagus, and in large quantities (30 to 50 milligrammes) weakens and slows the heart in the same way as piperidine. The action of coniine on the heart does not seem, however, to be stronger than that of piperidine. I have been unable to confirm the statement that coniine or any of its homologues possesses any action whatsoever on the central nervous system in frogs. The general symptoms do not seem to call for the hypothesis of any such action, for exactly the same symptoms may be produced by small doses of curara. The presence of any central action is, however, always difficult to prove or disprove when the peripheral motor apparatus is thrown out of action by the drug, and it was only after a long series of experiments that I could satisfy myself that no such central action comes into play in coniine poisoning in cold-blooded animals. My method was to prepare two frogs by severing the cord in the region of the medulla and ligaturing the left iliac artery in each. The reflex time was taken from each hind leg of each frog repeatedly, and coniine was then injected into one. The reflex time was afterward taken at intervals for several hours.

Example.

10.30—Average reflex time: Esculenta A, left leg, $1\frac{4}{5}$ second; right leg, $1\frac{2}{5}$ second. Esculenta B, left leg, $1\frac{1}{5}$ second; right leg, $1\frac{1}{5}$ second.

10.50—Twenty milligrammes of coniine injected into the abdominal lymph sac of B.

11.30—Average reflex time: Esculenta A, left leg, $1\frac{2}{5}$ second; right leg, 1 second. Esculenta B, left leg, $1\frac{3}{5}$ second; right leg, $1\frac{4}{5}$ second.

The right leg of B did not move at all now on irritation or on stimulation of the cord. The time given for its movement is that for the cross reflex—i. e., the time between the irritation of the right leg and the movement of the left leg.

12.30—Average time: Esculenta A, left leg, 2 seconds; right leg, $1\frac{4}{5}$ second. Esculenta B, left leg, $2\frac{4}{5}$ seconds; right leg, $4\frac{1}{5}$ seconds (cross reflex).

The reflex time was found, as a general rule, to be somewhat increased after the injection of coniine, but this increase is so small compared with that caused by even minute doses of any of the narcotics of the fatty series that it becomes a question whether it is really due to a direct action of coniine on the spinal cord. In any case the change seems to me insufficient to justify coniine being credited with the marked narcotic properties which were formerly ascribed to it. Kölliker,* Guttmann,† Prevost,‡ and others also deny the presence of any marked depressant action. On the other hand, in experiments in which the aorta was ligatured and the lower limbs, therefore, kept free from the poison, no movements were observed indicating a stimulant action on the cord such as that described by Harnack and Meyer.#

I came to the conclusion, then, that coniine differed from piperidine only in its much more powerful action on the motor-nerve ends, and that a series may be formed of piperidine, *a*-pipecoline, *a*-ethylpiperidine, and coniine, the members of which differ only in the strength but not in the kind of action on the frog. The increase in strength in the last three members can be easily estimated. Thus ethylpiperidine is two or three times as poisonous as methylpiperidine. Piperidine is undoubtedly much less poisonous than any of the others, but the difference can not be stated numerically from my experiments, as complete paralysis can not be obtained by its action.

* Virchow's *Archiv*, x.

† *Berl. klin. Wochenschr.*, 1866.

‡ *Arch. de Physiologie*, 1880.

Archiv f. exp. Path. und Pharm., xii, p. 393.

Putting the toxicity of pipecoline at 100, we find, then, that that of ethylpiperidine and propylpiperidine would be represented by 200 and 400. Guerber considers coniine eight times as poisonous as piperidine, and accepting this estimate, the toxicity of piperidine would be in my scheme 50. Thus the series may be represented,

Piperidine ($C_5H_{11}N$)	equals	50
Methylpiperidine ($CH_3C_5H_{10}N$)	equals	100
Ethylpiperidine ($C_2H_5C_5H_{10}N$)	"	200
Propylpiperidine ($C_3H_7C_5H_{10}N$)	"	400

or, while the methyl groups increase in arithmetical progression, the toxicity increases in geometrical. Guerber has formulated this law as expressing the toxicity of various members of the lupetidine series, which are also compounds of piperidine. He finds that while the lower members of the series conform to it, the higher ones prove exceptions because they possess a secondary action on the central nervous system. The cause of this may be that while in the lower members of the series the action of the piperidine radicle is the determining factor in the toxicity, as the number of methyl groups becomes greater they begin to have an action of their own as fatty narcotics. As far as I have gone in this series, no such narcotic action can be, I think, determined, but the higher members may also be endowed with it.